JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXXII WASHINGTON, D. C., MAY 15, 1926 No. 10

A PHYSIOLOGICAL STUDY OF THE GROWTH OF THE MEDITERRANEAN FLOUR MOTH (EPHESTIA KUEHNIELLA ZELLER) IN WHEAT FLOUR[1]

By CHARLES H. RICHARDSON[2]

Entomologist, Fruit-Insect Investigations, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

Although much valuable information has been gathered in regard to the natural food substances necessary for the growth of many species of insects, there is still a need for additional information on the quantity and quality of proteins, carbohydrates, fats, salts, vitamins, and other substances necessary for their growth and maintenance. Such studies would be of interest from the standpoint of comparative physiology, and would give knowledge of value in the control of insect pests. Poisoned food preparations are now used as baits to control such insects as cockroaches, crickets, grasshoppers, cutworms, house flies, and ants, and it is quite possible that investigations of the food requirements of these and perhaps other insects would lead to the adoption of baits of greater efficiency than those now employed. The present results are offered as a contribution to some of these problems.

The modern knowledge of vitamins has been largely derived from feeding experiments on mammals and birds, especially on the rat, the guinea pig, and the pigeon, but within recent years the investigations have been extended to include certain protozoa, insecta, batrachia, and additional species of birds and mammals. Vitamins or vitaminlike substances have been found also in certain bacteria, yeasts, and higher plants (*12*)[3]. The chemical nature of vitamins is still in doubt. They are recognized by their solubility in various reagents, their curative effects on animals suffering from certain deficiency diseases, and their stimulating powers on growth. They occur in nature associated with other substances, and it is improbable

[1] Received for publication July 14, 1925; issued May, 1926. The investigation reported here was begun in the fall of 1917 and continued with some interruptions until May, 1921. A brief summary of the results was presented before the Entomological Society of America at Toronto, Canada, December 28, 1921; and the results have also been referred to by Harrow (*16, p. 115*). Publication of the present paper has been delayed through unavoidable circumstances in connection with postwar conditions.

[2] The writer wishes to acknowledge his indebtedness to Prof. William J. Gies, of Columbia University, for his guidance and suggestions; to G. F. White, of the Bureau of Entomology, who has rendered much assistance in the control of a protozoan disease of the moth larvae; and to P. W. Whiting, of the University of Maine, T. J. Headlee, State entomologist of New Jersey, and Z. P. Metcalf, of the North Carolina State College of Agriculture and Engineering, who have furnished the writer entomological material.

[3] Reference is made by number (italic) to "Literature cited," p. 927.

that they have yet been isolated in a pure condition, although several highly concentrated vitamin preparations have been reported.

Not all animals respond equally to a given concentration of a vitamin-containing preparation, the amounts necessary for growth, maintenance, and reproduction varying considerably, in some cases, from species to species. For instance, rats and mice and many species of birds seem to grow normally in the absence, or at least with only a very low concentration, of vitamin C, whereas guinea pigs rapidly succumb unless this vitamin is present in the food.

Of the vitamins now recognized as essential for the growth of higher animals, probably only two exist in an active condition in the wheat kernel, namely, vitamin A and vitamin B. Dry cereals apparently do not possess vitamin C in an active state.

VITAMIN STUDIES ON INSECTS

The studies on insects which have a bearing on the influence of vitamins in nutrition have largely been made on the fruit fly *Drosophila melanogaster* (*ampelophila*) Loew. Loeb and Northrop (*20*) raised 12 successive generations of Drosophila under aseptic conditions upon a medium of sterile yeast, citric acid, and water. Casein, edestin, egg albumin, milk, or a mixture of amino acids, when supplemented with a salt mixture and sucrose, did not permit growth to the pupal stage. The substance in yeast which promoted growth was fairly heat-stable. When the yeast was extracted with hot or cold alcohol it could not be utilized as food, nor would a mixture of the extracted yeast and the alcoholic extract support the growth of the larvae. The addition of butter, nucleic acid, thymus, or thyroid extract to the culture medium likewise did not permit growth. Those writers concluded that "the necessary substance must be different from that needed for pigeons, rats, and other warm-blooded animals." Northrop (*25*) believes that the yeast plant supplies Drosophila larvae with growth-promoting substances similar to vitamins, which the plant is able to synthesize from simple chemical compounds of known structure. He obtained normal growth and development with such glandular tissues as liver, pancreas, and kidney, and also with dead adult Drosophila, but spleen, heart muscle, skeletal muscle, blood, adrenal, thyroid, and thymus glands, testes, tumor tissue from a mouse, and tethelin resulted at most in abnormal growth. In a later study Loeb and Northrop (*21*) found that while the larvae required yeast, the adult flies could live for a relatively long time on a medium consisting of beef broth, agar, peptone, egg, and glucose. It was thought that the vital substances found in yeast were not required for maintenance, or were needed in such small amounts that they could be supplied by the hydrolytic processes which take place in the cells of the adult. Northrop (*26*) observed that a deficiency in yeast caused a retardation of growth during the larval period, but the length of life of the adult fly was not affected.

Guyénot (*15*), in an article which is the culmination of an extensive series of experiments on the growth of *Drosophila melanogaster*, concludes that sterilized yeast can be replaced by an artificial medium consisting of salts, peptone, lecithin, and an undetermined substance present in the alcoholic extracts, bouillons, and autolysates of yeast and liver tissue. The substance in yeast which is indispensable for

the life of Drosophila is soluble in water, insoluble in cold 100 per cent alcohol, soluble in boiling 100 per cent alcohol, from which it is precipitated on cooling, and soluble in 90, 80, and 70 per cent alcohols. It resembles in certain respects the water-soluble vitamin B necessary for the normal life of higher animals.

Baumberger (*3*) succeeded in rearing adult *Drosophila melanogaster* upon a medium consisting of yeast nucleoprotein, carbohydrates, and salts. He believes that "the larvae are dependent on the nucleoprotein of yeast for special substances necessary in their growth" and that lecithin is unnecessary for normal development. The nucleoprotein was washed first with acid alcohol, then with neutral alcohol, and finally extraced with ether to free it of impurities.

Bacot and Harden (*2*) grew larvae of *Drosophila melanogaster* aseptically on a basal diet of purified casein, starch, sucrose, agar, and a salt mixture, to which the various vitamin preparations were added. In the presence of butterfat (vitamin A) and yeast extract (vitamin B), growth occurred irrespective of the presence or absence of lemon juice (vitamin C). When butterfat was added, growth occurred if yeast extract was present, but failed in its absence. Here also growth seemed not to depend upon the presence or absence of lemon juice. In media containing yeast extract with or without lemon juice, growth occurred when as little as two drops of butterfat was added, but did not take place in its absence. When casein was omitted from the diet, the nitrogen furnished by the yeast extract was insufficient for the growth of the larvae. Vitamin B from wheat germ also permitted growth in the presence of two drops of butterfat. Those writers concluded that complete development of Drosophila requires the presence of vitamin B, and very small quantities, if any, of vitamin A. The antiscorbutic vitamin (C) is not essential for growth and development.

Glaser (*13*) studied the effect of food on the longevity and reproduction of *Musca domestica* L., *Stomoxys calcitrans* L., and *Lyperosia irritans* L. He mentions briefly the intestinal bacteria of adult flies as a possible source of vitamins during the adult stage. Elsewhere the same writer describes experiments (*14*) in which larvae of *Drosophila melanogaster* grew and became adults that laid many fertile eggs on sterile media to which small quantities of fresh sterile orange and grapefruit juices were added daily. It was thus shown that Drosophila can live on fruit substances without the aid of microorganisms, and in the absence of products liberated from the dead bodies of microorganisms.

Wollman (*35*) grew larvae of *Calliphora vomitoria* aseptically upon brain tissue which had been sterilized in an autoclave at 130° C. for 45 minutes. He advanced these three hypotheses to account for this fact: Either the vitamin of the brain tissue was not destroyed at the temperature used, or the larvae did not require vitamins for growth, or they may have synthesized vitamins from materials in the medium. In another article (*36*) he proved that a temperature of 134° to 135° C. for 1½ hours (using an autoclave) did not destroy all the vitamin in brain tissue necessary for the growth of rats, and that Calliphora larvae grown aseptically on brain tissue sterilized at a high temperature furnished an appreciable amount of vitamin when fed to young rats. He concludes that the larvae do not produce vitamin, but accumulate and concentrate the vitamin which they obtain from the sterilized brain tissue. More recently Wollman (*37*) has been able

to grow larvae of the house fly (*Musca domestica* L.) aseptically upon horse manure sterilized at a low temperature.

Portier (*31*) reared larvae of *Tenebrio molitor* on a mixture of bran and flour sterilized at 130° C. in an autoclave for 45 minutes. He interprets his results as follows: Symbiotic microorganisms, present in foods, are responsible for the synthesis of the special substances necessary for life. Certain species of insects, of which Tenebrio is one, have normally a supply of symbiotic microorganisms in the epithelial cells of the intestine. The symbionts are passed from the parent insect to its offspring by means of the egg. Other species of animals derive their necessary symbiotic microorganisms from the food. When the food is sterilized at a temperature sufficiently high to kill the symbionts, the species like Tenebrio which possess symbiotic organisms within the intestinal epithelium grow normally, while those species not so endowed cease to grow and ultimately die.

Aeppler (*1*) investigated the vitamin content of the food of the larval honeybee, *Apis mellifera* L. It did not contain sufficient vitamin A to support the growth of young rats at the normal rate. Vitamin B was present, however, in amounts sufficient for the normal growth of rats. Since it has been shown by Hawk, Smith, and Bergeim (*17*) that honey contains very little vitamin B, it is believed that the amount present in the larval food is derived from the pollen[4].

From the foregoing publications it seems to be an established fact that vitamin B is necessary for the normal growth of certain insects, particularly *Drosophila melanogaster*. Baumberger's results (*3*) are rather opposed to this view, but there is a possibility that the yeast used by him contained sufficient vitamin B, even after purification, for the growth of the larvae. Yeast is one of the richest known sources of this vitamin, and, according to Osborne, Wakeman, and Ferry (*30*), certain proteins are freed from it with difficulty.

The rôle of vitamin A is not so clearly defined, but from the work of Bacot and Harden (*2*) there is reason for believing it a necessary constituent of the food of insects. The present work lends strong support to this view. The investigations on Drosophila also show that not more than traces of fats in any form are necessary in the diet of this insect.

The information at present available leads to the conclusion that vitamin C is not essential for the normal growth and development of insects.

LIFE HISTORY OF EPHESTIA KUEHNIELLA

The Mediterranean flour moth belongs to the lepidoterous family Pyralidae, and is the commonest of the species of Ephestia found in North America. It occurs wherever flour and other cereal foods are

[4] The report of an interesting study of the nutrition of the flour beetle (*Tribolium confusum*) by Chapman (*7*) has recently appeared. It is found that the lower grades of wheat products (low-grade flour and bran) slightly retard growth and metamorphosis, whereas rice flour made from polished rice slightly retards growth but has a marked effect upon metamorphosis. When all the embryo is removed, the effect of the rice flour is still more pronounced. Oat, rye, and corn flours also retard growth slightly and metamorphosis more, while barley flour resembles the best grades of wheat flour in its effect. The effects of a number of synthetic mixtures on growth and transformation are also given. Chapman concludes that wheat embryo is more satisfactory for growth and transformation than the other substances tried, that vitamin B from wheat embryo does not seem to supplement deficient diets, and that the requirements for growth, in the sense of increasing size, are less exacting than for maturity or transformation. Vitamin A does not seem to have been present in any of these food mixtures, except in one which contained wheat embryo.

stored or manufactured, and often causes serious damage in mills and warehouses.

Fertilized females generally lay their eggs from 24 to 48 hours after emergence. Females laying infertile eggs and virgin females require a longer time for oviposition than is usual for fertile moths. The eggs are normally laid during the night and are placed in the food material, but when food is absent they may be scattered indiscriminately about the walls of the container in which the moths are placed. According to Burkhardt (*5*) the female lays between 150 and 200 eggs. Small females lay fewer. Under the conditions of these experiments, the incubation period varied from 4 to 11 days, depending on the temperature. During the cooler part of the year the average period was 6 days.

The newly-hatched larva measures about 1 mm. in length, and weighs about 0.023 mgm. (mean weight of 150 larvae). It begins feeding at once, and spins a silken tube as it works its way through the food substance. The amount of silk produced gives a rough idea of the extent to which growth has taken place. In normal food the larvae attain a length of about 12 mm. and a weight of about 24.5 mgm. before pupation. The growth rate varies with the temperature, kind of food, and possibly other factors.

Various observers have stated the duration of the pupal stage as from 8 to 16 days at ordinary summer temperature, but under adverse conditions of temperature it may be greatly extended.

The moth lives but a short while after emergence, and apparently it takes no food during that time. The mean length of life under laboratory conditions for 30 males was 9.5 days and for 34 females was 8.5 days, while the maximum duration of life was 14 and 15 days, respectively.

Under conditions of apparently normal nutrition and environment, great variations in the fertility of the moths have been observed. Whiting (*34*) found that usually not more than 5 or 10 per cent of the pairs of moths were fertile under experimental conditions, though occasionally the percentages rose to as high as 50, 60, or even 70. In one instance the writer observed a fertility of 90.5 per cent in 42 pairs under experimental conditions, but often the ratio is lower than this. In view of this fact, the question of the effect of food upon fertility has not been considered at this time.

The sex ratio of moths, as based on 1,382 individuals, was found to be 47.5 per cent males and 52.5 per cent females.

Although the moths are easily reared in a small space, considerable care is necessary to prevent the spread of disease among them. The larvae are subject to infection by a protozoan parasite which often causes a heavy mortality and may at times kill all the larvae in a bottle. However, the larvae which complete growth and transformation in the infested bottles emerge as moths in approximately normal time. Sterilization of the bottles in which the larvae are grown, and selection of moths from disease-free cultures, will exclude the parasites to a very large extent.

FOOD OF EPHESTIA KUEHNIELLA LARVAE

Although Ephestia larvae are most frequently observed in wheat flour, they are able to live in many other foods. They have been found in wheat bran, entire wheat kernels, rye flour, buckwheat

flour, corn meal, entire corn kernels, rice flour, rolled oats, cottonseed meal, prepared cereal foods, crackers, cakes, chestnuts and other nuts, cocoa beans, chocolate, dried fruits, jelly cubes, nests of the bumblebee, hives of the honeybee, and dead Ephestia and Tortrix moths. The writer has reared moths from commercial barley flour, flour made from entire hemp seed, and flour from entire field-pea seed. In two experiments with this pea flour the time taken for the first moth to emerge was 94 and 104 days, respectively, an abnormally protracted period of growth when compared with the life cycle in entire-wheat flour.

A number of attempts were made to rear moths from flour made of entire Georgia velvet beans, navy beans, lima beans, and soybeans. The moths seemed to oviposit readily in these flours, but the young larvae invariably died within a few weeks after hatching. In not one instance did a larva attain even half normal growth. It is interesting to note that McCollum, Simmonds, and Pitz (*23*) found that large quantities of navy beans (*Phaseolus vulgaris*) were injurious to rats, partly because of the low nutritive value of the proteins in the beans. Later McCollum, Simmonds, and Parsons (*24*) found that the protein of peas (*Vicia sativa*) was also inadequate for optimum growth in rats. More recently, Johns and Finks (*19*) have found that the navy-bean proteins are deficient in cystine, and that rats show only normal growth on diets containing all the protein from navy beans, provided such diets are supplemented with this amino acid. This explanation may also hold in the case of Ephestia, but that it can not be applied to insects generally is indicated by the well-known bean and pea feeding habits of the larvae of certain Coleoptera such as *Bruchus obtectus* and *B. quadrimaculatus*.

EXPERIMENTAL METHODS

Clean, sound, whole wheat was reduced to fine flour in a small hand mill. None of the coarser bran was removed, and the mositure content of the flour was not altered in any way before treatment with the solvents.

The highly milled (patent) wheat flour was a well-known commercial brand of white flour.

The Soxhlet apparatus used for most of the extractions had a capacity of 100 c. c. From 35 to 45 gm. of flour, placed in a fat-free paper extraction thimble, were treated at a time. A small number of extractions were made with a 200 c. c. Soxhlet, but the amount of flour used was the same as with the smaller apparatus. The heat was supplied by a water bath.

The methods of preparation or extraction of the food used in the individual experiments are described in the legends accompanying the figures.

The essential differences between extraction in a Soxhlet apparatus and extraction at room temperature are the higher temperature and the more frequent change of solvent in the former method. A solvent which has little or no effect on the growth value of the flour, ether for example, removes the growth-promoting substance in a much shorter time in a Soxhlet apparatus than at room temperature with several changes of solvent. On the other hand, U. S. P. chloroform when used without special purification in a Soxhlet apparatus

appears to produce destructive changes in the flour, making it unfit for growth, whereas at room temperature no such effects have been observed. It should be noted that the temperature of the solvent surrounding the flour in a Soxhlet apparatus is always below the boiling point of the solvent.

The rate of growth of Ephestia larvae was studied by comparing the length of the life cycle on an experimental food with that on a standard, i. e., entire-wheat flour. The work was done in a laboratory room. Such climatic factors as temperature, moisture, and light were not closely regulated, since apparatus for this was not available. It is believed, however, that all the experiments were affected in nearly the same degree by any fluctuations of climate that occurred during their course.

The life cycle was determined from the day on which moths or young larvae were isolated in a bottle containing the food under investigation. Only moths which had emerged within a 24-hour period were used. In the earlier experiments, a number of moths, usually four or five, were selected at random without regard to sex, but later one or several pairs of moths were employed. Unless it could be positively determined that larvae had hatched from eggs laid by the moths, the experiment was rejected. Many of the later experiments were started with a number of newly hatched larvae, usually 20, isolated on 5 gm. of flour, which furnished ample food for this number. They were obtained by isolating a pair of moths in a bottle and carefully removing the newly hatched larvae with a camel's-hair brush. Larvae hatching during 24 or 48 hours, rarely 72 hours, were selected for this purpose. In order to reduce them all to a comparable age basis, the larvae which hatched within a 48 or 72-hour interval were considered as one and two days older, respectively, than those hatching within the 24-hour interval, such time being added to the life cycle.

Daily records of the number of moths that emerged were taken, and from them the time (in days) to first emergence, last emergence, and average emergence was obtained. The last figure is the sum of the products of the life cycle in days and the number of moths emerging, divided by the total number of moths emerged.

Larvae were reared in several types of bottles. In the later experiments where the progeny of a single pair of moths or young larvae were reared, a wide-mouthed bottle 80 mm. high and 57 mm. in diameter at the base, was employed extensively. The bottles were stoppered with cotton plugs which were easily removed, but often the full-grown larvae worked their way into the cotton and pupated there. Moths have difficulty in emerging from the pupal cases in cotton and frequently die before they can free themselves. Such dead moths were always considered as normally emerged individuals.

GROWTH OF LARVAE IN NORMAL ENTIRE-WHEAT FLOUR

In order that a check on the effect of environmental factors other than food might be obtained, a control experiment with normal entire-wheat flour was run parallel to each series of experiments in which treated flour was used. The results are expressed in curves

(fig. 1) showing the length of the life cycle (obtained by the method of moving averages) for any time of the year at Washington, D. C., under the conditions governing these experiments.

Similar results were obtained for a shorter period at New York City (fig. 2). The ordinates represent the time (in days) from the isolation of moths or young larvae upon the normal flour to the emergence of moths of the new generation. The abscissae give the time of the year at which the experiments were started. Curve A (fig. 1) gives the length of the life cycle of the first moth to emerge; curve B, the average length of the life cycle of all moths; while curve C is the life cycle of the last moth to appear.

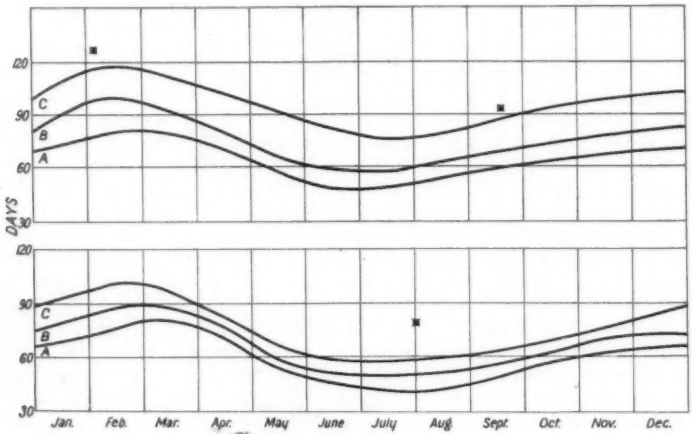


FIG. 1

Growth rate of Ephestia larvae in entire-wheat flour, as determined by the length of the life cycle. Period, June, 1919, to June, 1920. Washington, D. C. Room temperature, subject to daily and seasonal fluctuations.

Upper curve.—Progeny from one or several pairs of moths placed on 10 to 15 gm. flour. (Based on 60 experiments.)

Lower curve.—Progeny from young larvae (usually 20) placed on 5 gm. flour. (Based on 99 experiments.)

A represents the length of the life cycle of the first moth which emerged, B the average time (in days) of emergence of all moths, and C the length of the life cycle of the last moth to appear.

These curves are derived from data smoothed by the method of moving averages.

The life cycle for progeny from pairs of moths grown in normal flour varied from a minimum of 46 days in summer to 85 days in winter. The average emergence varied from 54 to 105 days, and the last emergence from 64 to 124 days. Moths from newly hatched larvae required from 41 to 85 days from the first emergence, 48 to 100 days for the average emergence, and 54 to 114 days for the last emergence. These extreme deviations are not expressed in the curves in Figure 1 because of the smoothing to which the curves have been subjected.

The values for first emergence and average emergence give quite smooth curves by the method of moving averages. Both are valuable indices of the growth rate. The emergence of the last moth is more variable, and the curves (C) have been subjected to considerable smoothing.

The difference between experiments started with moths and those started with young larvae lies practically in the incubation period

of the eggs, which varies from 4 to 11 days, depending on the time of the year.

The number of moths which emerged from entire-wheat flour when the experiment was started with moths was variable, since there was often a marked difference in the number of eggs laid as well as in the proportion of those eggs which hatched. Of the experiments started with a definite number of larvae 24 or 48 hours old, involving 1,165 individuals, 76 per cent pupated and emerged as moths. A part of this mortality may have been due to injuries received while being transferred on the camel's-hair brush, but it is doubtful whether all of it can be explained in this way.

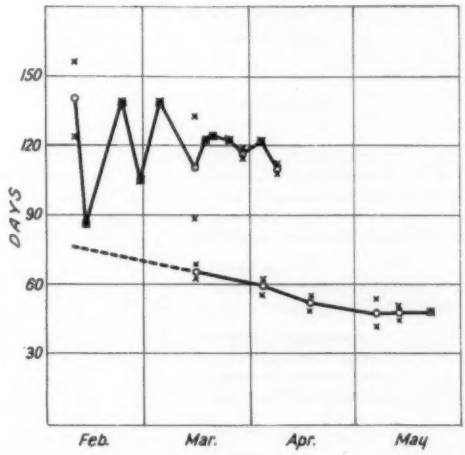


FIG. 2

Upper curve.—Days to appearance of first moth, in 18 experiments. Progeny from 4 or 5 moths placed on 10 gm. highly milled (patent) wheat flour February 8 to April 8, 1918. New York City.
Lower curve.—Days to appearance of first moth in 13 experiments. Progeny from 5 moths placed on 10 or 15 gm. normal entire-wheat flour, March 15 to May 22, 1918. New York City.
X represents a single observation; O represents the average first emergence for two or more experiments started on a given date.

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH ETHER

Entire-wheat flour was extracted with anhydrous ether at room temperature, and also in a Soxhlet apparatus, for varying lengths of time.

The effect of flour, extracted for 24 hours at room temperature, on the life cycle of Ephestia is shown in Figure 3. Experiment 2, without change of ether, is comparable to experiment 3, in which there was one change of ether during the 24 hours' extraction, and is not very different from experiments 4 and 5, in which there were two changes of ether. Although a comparison of the average time of emergence of moths in the 24-hour-extracted flour indicates a more extended period of growth than in the control, it is apparent that only a relatively small amount of the growth-promoting substance was removed.

Three or four hours' extraction in a Soxhlet apparatus does not remove much of the growth-promoting substances. Experiments 18 to 22, begun in June from pairs of moths, lost most of their larvae through disease, and all the larvae in the control were destroyed. However, they are comparable to experiments 197 to 199 (started in February from newly hatched larvae) when the controls of the respective series are considered and allowance is made for the small emergence in experiments 18 and 21. The control A was obtained from the curves in Figure 1.

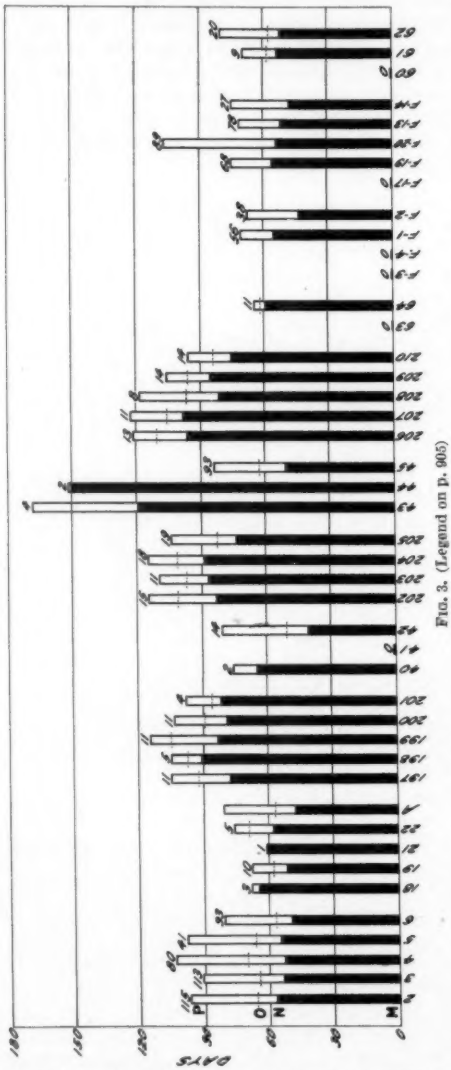


FIG. 3. (Legend on p. 905)

A 7-hour extraction in a Soxhlet apparatus effected a greater removal of the ether-soluble growth-promoting substance, but enough of the material still remained for growth. Experiments 40 and 41, commenced the last of July, gave much poorer growth than experiments 202, 203, and 204, all of which were started in February.

A 10-hour extraction in a Soxhlet apparatus likewise did not remove all of the growth-promoting substance. In experiments 206 and 207, the life cycle was markedly longer than in the control, experiment 210; when the extracted material was returned quantitatively to the extracted flour, the growth-rate (experiments 208 and 209) approached that in the control, but was exceptional in that it was still slow. Experiments 43 and 44 gave quite different results in regard to the length of the life cycle, and illustrate the variation one may expect in this kind of experimental work.

Growth generally ceased or was greatly retarded in flour that had been extracted in a Soxhlet apparatus for 15 hours or more. The best results were obtained when the Soxhlet flask was heated sufficiently to permit six ether changes per hour. In one experiment (63, fig. 3), a complete removal of the growth-promoting substance was apparently accomplished in 12 hours, but as this was not repeated it may be doubted whether growth would invariably cease in flour extracted for this length of time.

When only a few weeks old, most of the larvae died in the highly extracted flour; and the few that survived for a longer period usually failed to reach the pupal stage. If, however, the extracted material

EXPLANATORY LEGEND FOR FIGURE 3

The solid black vertical lines MN indicate the length of the life cycle (in days) from the introduction of moths or larvae in the bottle to the emergence of the first moth of the new generation. O represents the average emergence (in days) of all moths; and P the number of days to the emergence of the last moth in the experiment. The number at the top of each column represents the number of moths that have emerged in the experiment. The last line in practically every group represents either the control experiment or a duplicate of it.

2. 50 gm. entire-wheat flour extracted with 200 c. c. anhydrous ether, at room temperature, for 24 hours. 2 males, 3 females, isolated on 15 gm. flour June 4; 1 male added June 5.
3. 50 gm. entire-wheat flour extracted at room temperature, 5 hours, with 100 c. c. anhydrous ether; and 19 hours with another 100 c. c. ether. 1 male, 3 females, isolated on 15 gm. flour June 4; 1 male added June 5.
4. 50 gm. entire-wheat flour extracted at room temperature, 5 hours with 100 c. c. anhydrous ether, 16 hours with 100 c. c. ether, and 3 hours with 100 c. c. ether. 3 males, 3 females, on 15 gm. flour, June 5.
5. Duplicate of 4.
6. (Control.) 3 males, 3 females, isolated on 15 gm. normal entire-wheat flour, June 5.
18. 36 gm. entire-wheat flour extracted 3 hours, Soxhlet, with anhydrous ether. 3 males, 3 females, isolated on 15 gm. flour, June 25.
19. Duplicate of 18.
21. 36 gm. entire-wheat flour extracted 4 hours, Soxhlet, with anhydrous ether. 3 males, 3 females, isolated on 15 gm. flour, June 25.
22. Duplicate of 21. Larvae in experiments 18, 19, 21, and 22 were all badly diseased, which doubtless accounts for the small number of moths produced.
A. (Control.) Taken from curves of Figure 1. Larvae in original control died of disease.
40. 40 gm. entire-wheat flour extracted 7 hours, Soxhlet, with anhydrous ether. 20 larvae about 18 hours old isolated on 10 gm. flour, July 31.
41. Duplicate of 40.
42. (Control.) 20 larvae about 18 hours old isolated on 10 gm. normal entire-wheat flour, July 31.
43. 40 gm. entire-wheat flour extracted 10 hours, Soxhlet, with anhydrous ether. 2 males, 2 females, isolated on 15 gm. flour, August 4.
44. Duplicate of 43.
45. (Control.) 2 males, 2 females, on 15 gm. normal entire-wheat flour, August 4.
60. 33 gm. entire-wheat flour extracted 16¼ hours, Soxhlet, with anhydrous ether. 2 males, 2 females, isolated on 16 gm. flour, August 24.
61. 16 gm. flour from 60 mixed with half of the ether extract, and the ether evaporated at room temperature. 2 males, 2 females, isolated on this flour, August 24.
62. (Control.) 2 males, 2 females, isolated on 16 gm. normal entire-wheat flour, August 24.
63. 40 gm. entire-wheat flour extracted 12 hours, Soxhlet, with anhydrous ether. 1 male, 1 female, isolated on 15 gm. flour August 28; and 1 male, 1 female, isolated August 29. Larvae hatching from the eggs laid in this flour died in less than 3 months.
64. (Control.) 1 male, 1 female, isolated on 15 gm. normal entire-wheat flour, August 30.
F-1. (Control.) 5 moths isolated on 10 gm. normal entire-wheat flour, May 6.
F-2. Duplicate of F-1.
F-3. 40 gm. entire-wheat flour extracted 15 hours, Soxhlet, anhydrous ether. 5 moths isolated on 10 gm. flour, May 6.
F-4. Duplicate of F-3.
F-13. (Control.) 5 moths isolated on 15 gm. normal entire-wheat flour, May 13.
F-14. Duplicate of F-13.
F-17. 10 moths isolated on 15 gm. flour extracted 15 hours (see F-3), May 13.
F-19. 40 gm. entire-wheat flour extracted 12 hours, Soxhlet, with anhydrous ether, after which the ether extract was returned to the extracted flour quantitatively. 5 moths isolated on 15 gm. flour, May 13.
F-20. Duplicate of F-19, except that 13.5 gm. instead of 15 gm. flour was used.
197. 43.5 gm. entire-wheat flour extracted 4 hours, Soxhlet, with anhydrous ether. 20 larvae about 24 hours old isolated on 5 gm. flour, February 20.
198. Duplicate of 197.
199. Duplicate of 197.
200. (Control.) 20 larvae about 24 hours old isolated on 5 gm. entire-wheat flour, February 20.
201. Duplicate of 200.
202. 41.9 gm. entire-wheat flour extracted 7 hours, Soxhlet, with anhydrous ether. 20 larvae about 24 hours old isolated on 5 gm. flour, Feburary 21.
203. Duplicate of 202.
204. Duplicate of 202.
205. (Control.) 20 larvae about 24 hours old isolated on 5 gm. normal entire-wheat flour, February 21.
206. 44 gm. entire-wheat flour extracted 10 hours 10 minutes, Soxhlet, with anhydrous ether. 20 larvae about 24 hours old isolated on 5 gm. flour, February 24.
207. Duplicate of 206.
208. Half of the flour from 206 with half of the ether extract returned to it quantitatively, and the ether evaporated at room temperature. 20 larvae about 24 hours old isolated on 5 gm. flour, February 24.
209. Duplicate of 208.
210. (Control.) 20 larvae about 24 hours old isolated on 5 gm. normal entire-wheat flour, February 24.

containing the growth-promoting substance was returned to the residue, growth similar to that in the normal flour was obtained (F–19, F–20, 61, fig. 3), indicating that it was largely a matter of

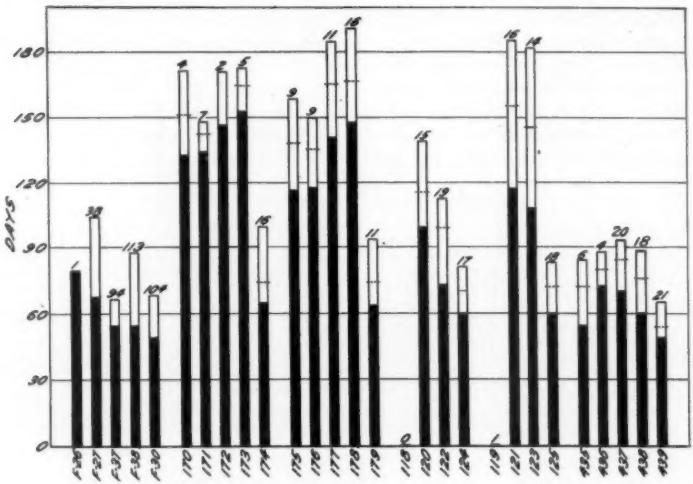


FIG. 4

F–26. 40 gm. entire-wheat flour extracted 15 hours, Soxhlet, with 95 per cent alcohol (redistilled from CaO). 10 moths placed on 15 gm. flour, May 22.

F–27. Duplicate of F–26.

F–30. (Control.) 10 moths placed on 15 gm. normal entire-wheat flour, May 22.

F–37. 40 gm. entire-wheat flour extracted 15 hours, Soxhlet, with 95 per cent alcohol (redistilled from CaO), after which the alcoholic extract was mixed with the extracted flour and the solvent evaporated at room temperature. 5 moths placed on 15 gm. flour, May 31.

F–38. Duplicate of F–37.

118. 40 gm. entire-wheat flour extracted 15 hours with anhydrous ether, and then for 10 hours with 95 per cent alcohol. Half the alcoholic extract was mixed with one-third of this extracted flour, and the solvent evaporated off at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, November 5.

119. 20 larvae 24 to 36 hours old placed November 30 and December 1 on 5 gm. of the flour used in 118. One moth emerged; life cycle 275 days.

120. One-third the flour extracted with both ether and alcohol (118) was mixed with half of the ether extract. The ether was removed at room temperature. 17 larvae about 48 hours old placed on 5 gm. flour, November 5.

121. 20 larvae about 24 hours old placed December 1 on 5 gm. of the flour used in 120.

122. One-third of the flour extracted with both ether and alcohol (118) was mixed with half of the ether, and then with half of the alcoholic extracts, the solvents being removed at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, November 6.

123. 20 larvae about 24 hours old placed December 1 on 5 gm. of the flour used in 122.

124. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, November 6.

125. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, December 1.

170. 41.8 gm. entire-wheat flour extracted 10 hours, Soxhlet, with absolute alcohol. 20 larvae about 24 hours old isolated on 5 gm. flour, January 8.

171. Duplicate of 170.

172. Half of the extracted flour from 170 mixed with half of the alcoholic extract, and the solvent evaporated off at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, January 8.

173. Duplicate of 172.

174. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, January 8.

175. 45 gm. entire-wheat flour extracted 15 hours, Soxhlet, with absolute alcohol. 20 larvae about 24 hours old placed on 5 gm. flour, January 8.

176. Duplicate of 175.

177. Half of the extracted flour from 175 mixed with half of the alcoholic extract; alcohol evaporated at room temperature. 20 larvae 24 to 48 hours old placed on 5 gm. flour, January 8.

178. Duplicate of 177, except that 25 larvae about 48 hours old were used.

179. (Control.) 18 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, January 8.

435. 50 gm. entire-wheat flour extracted with 200 c. c. 95 per cent alcohol, for 24 hours, at room temperature. 4 c. c. extract equivalent to 1 gm. extracted flour. 20 larvae about 24 hours old placed on 5 gm. flour, July 26.

436. Same as 435.

437. 15 gm. extracted flour from 435 mixed with 60 c. c. of the alcoholic extract from same, and the solvent removed at room temperature. 20 larvae about 24 hoursold placed on 5 gm. flour, July 26.

438. Same as 437.

439. (Control.) 21 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, July 26.

the removal and not the destruction of a substance or substances, the absence of which was responsible for the growth failure in the extracted flour. Subsequent experiments have confirmed this view (442, 443, fig. 5).

It is believed that the failure of Ephestia larvae to grow in entire-wheat flour extracted with ether in a Soxhlet apparatus for 15 hours or more is due to the removal of vitamin A, the presence of which has long been known in the wheat kernel (*22*).

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH ACETONE

Acetone, when used at room temperature for a 24-hour period, was not an effective solvent of the growth-promoting substance in entire-wheat flour (29, 30, fig. 6). However, four hours' extraction in a Soxhlet apparatus removed enough of the substance to check the growth rate, but the food value of the residue plus the acetone extract was not equal to that of the original flour (213, 214, fig. 6). Flour subjected to continuous extraction for seven hours or more with acetone which had not been purified would not support growth at all, and the flour thus treated did not permit normal growth even when the extract was returned to it.[5]

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH BENZENE

In the series of experiments with benzene as the solvent, unless the flour was given a 15-hour period of extraction in a Soxhlet apparatus, removal of the growth-promoting substance was not sufficient to prevent growth (see fig. 7). The benzene treatment seemed to alter somewhat the food value of the flour, the life cycle always being longer on extracted flour to which the extract had been added than on normal flour. Extraction with benzene at room temperature for 24 hours apparently removed some of the growth-promoting substance, but the larvae in these experiments (26, 27, fig. 4) were so badly diseased as to leave some doubt concerning the efficiency of the solvent under these conditions.[6]

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH PETROLEUM ETHER

Entire-wheat flour was extracted with petroleum ether (b. p. 33° to 66°C.) for 9¾ and 15 hours, respectively. The results are shown in Figure 8. The larvae on extracted flour required a longer time for growth than those on the normal flour, but the removal of the growth-promoting substance was by no means complete. That all of the increase in time necessary for growth on the extracted flour was not due to the removal of a substance, however, is shown by experiments 143, 144, 151, and 152, in which growth was slower than in the con-

[5] Experiments recently completed with flour extracted with a specially purified acetone indicate that although some of the growth-promoting substance was removed, the growth value of the flour was not otherwise impaired by treatment with the solvent. The flour was extracted in a Soxhlet apparatus for 10 hours. Growth was retarded in the extracted flour, but was normal in the extracted flour plus the extract. The acetone U. S. P. was purified by allowing it to stand in contact with $CaCl_2$ for three days and distilling just before using.

[6] More recent experiments show that the extraction is not complete in 24 hours at room temperature. Flour extracted with purified benzene for 15 hours in a Soxhlet apparatus allowed slow growth, but the same flour with the extract returned had a normal growth value. The benzene C. P. was purified by shaking with concentrated sulphuric acid until the acid remained nearly colorless on standing overnight in contact with the solvent. The benzene was then shaken with sodium hydroxide solution, dried over calcium chloride, and distilled when ready for use.

trols, even when the extract was added to the extracted flour. The petroleum ether employed evidently had a deleterious action on the flour, under the conditions of these experiments.

Several experiments were made with the same lot of petroleum ether before the higher fractions were removed by distillation. Flour extracted at room temperature (50 gm. flour and 200 c. c. solvent) for 24 hours, gave 48 days for the first emergence, 58 days average emergence, and 66 days for the last emergence; or about what one would expect for normal flour. The experiment was started July 8. Ten hours' extraction in a Soxhlet apparatus allowed growth at the following rates: 57, 70, and 91 days, respectively, for the first, average, and last emergence; 55, 58, and 67 days were the corresponding rates for the control. The experiments were started August 14. The flour extracted for 15 hours in a Soxhlet apparatus, with the undistilled petroleum ether, gave rather unusual results. Growth in two duplicate experiments was as follows: 107 and 134 days, 160 and 172 days, and 199 and 205 days, respectively, for the first, average, and last emergence. The control gave 62, 69, and 75 days. This flour was extracted late in August and was not used until December 15. The unusually long period of growth probably resulted in part, at least, from the fact that the life cycle was extended into the coldest part of the year.

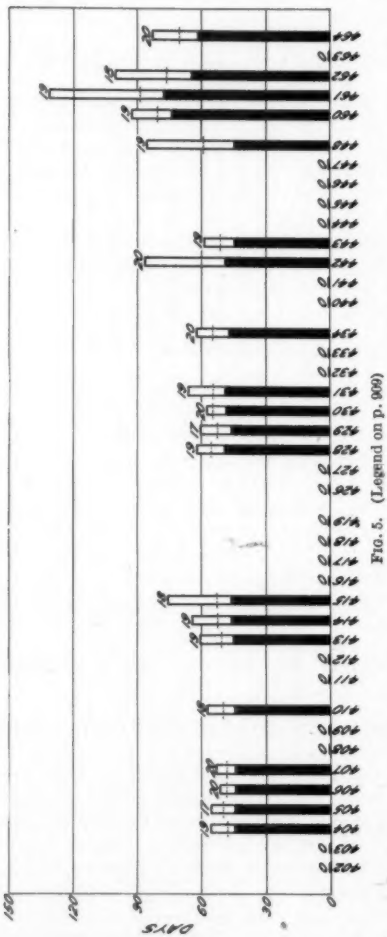


FIG. 5. (Legend on p. 909)

Since petroleum ether is a widely used fat solvent, the inference is that the growth-promoting substance incompletely extracted by it from entire-wheat flour is of the nature of vitamin A.

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH CHLOROFORM

In so far as the requirements of Ephestia larvae are concerned, removal of the growth-promoting substance was nearly complete when wheat flour was extracted with chloroform at room temperature for 24 or 25 hours. The growth rate of those moths which reached

maturity was considerably slower than that of the control moths. In experiment 37 (fig. 9), in which the larvae were badly diseased, a single moth emerged 20 days later than the single moth in the control, experiment 36. Six moths emerged from 40 larvae in experi-

EXPLANATORY LEGEND FOR FIGURE 5

402. 20 larvae about 48 hours old placed on 5 gm. entire-wheat flour extracted with chloroform U. S. P., at room temperature, for 28 hours, July 6. Flour was extracted as follows: 50 gm. flour treated with 100 c. c. solvent for 5 hours and 25 minutes, after which the solvent was removed on a Büchner funnel, and a second extraction was made with 100 c. c. solvent for 22 hours and 35 minutes. The chloroform again was removed on a Büchner funnel, and the flour was washed with two 25 c. c. portions of fresh chloroform, the washings being added to the extract. The solvent remaining in the flour was removed by evaporation at room temperature. 4.3 c. c. extract were equivalent to 1 gm. original flour.

403. Same as 402.

404. 10 gm. chloroform-extracted flour (see 402) mixed with 43 c. c. chloroform extract from the same. Solvent removed at room temperature. 20 larvae about 48 hours old placed on 5 gm. of this flour, July 6.

405. Same as 404.

406. 10 gm. chloroform-extracted flour (see 402) mixed with 10 c. c. of an ether extract of egg yolk (see 331, Fig. 13, for preparation of extract). Solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. of this flour, July 6.

407. Same as 406.

408. 10 gm. chloroform-extracted flour (see 402) mixed with 0.2 gm. olive oil dissolved in ether. 20 larvae about 48 hours old placed on 5 gm. flour, July 6.

409. Same as 408.

410. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour July 6.

411. 20 larvae about 24 hours old placed on 5 gm. entire-wheat flour extracted with chloroform U. S. P. for 24 hours, July 17. Flour extracted as follows: 50 gm. flour extracted with 100 c. c. chloroform, at room temperature, for 16 hours; solvent removed on Büchner funnel, and washed with 25 c. c. chloroform. A second extraction was made with 100 c. c. solvent, for 8 hours, after which the solvent was removed on a Büchner funnel as before and washed with 25 c. c. chloroform. Both washings were added to the extract. The flour was freed of chloroform by evaporation at room temperature. 4.24 c. c. extract equivalent to 1 gm. original flour.

412. Same as 411.

413. 10 gm. chloroform-extracted flour (see 411) mixed with 43 c. c. chloroform extract from the same. Solvent removed at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, July 17.

414. Same as 413.

415. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, July 17.

416. 10 gm. chloroform-extracted flour (see 411) mixed with 0.5 gm. butter dissolved in ether. 20 larvae about 24 hours old placed on 5 gm. flour, July 17.

417. Same as 416.

418. 10 gm. chloroform-extracted flour (see 411) mixed with 0.5 gm. lard dissolved in ether. 20 larvae about 24 hours old placed on 5 gm. flour, July 17.

419. Same as 418.

426. 20 larvae about 48 hours old placed on 5 gm. entire-wheat flour extracted with anhydrous ether for 18 hours, Soxhlet, July 30. Flour taken from a 43-gm. lot.

427. Same as 426.

428. 10 gm. ether-extracted flour (see 426) mixed with 8 c. c. of an ether extract of egg yolk. Solvent evaporated at room temperature. The egg-yolk extract was prepared from air-dried egg yolk extracted six times with boiling ether. 1 c. c. extract contained 0.025 gm. ether-soluble substance. 20 larvae about 48 hours old placed on 5 gm. flour, July 30.

429. Same as 428, except larvae were about 24 hours old and the experiment was started July 31.

430. 10 gm. ether-extracted flour mixed with 24 c. c. of the above ether extract of egg yolk. 20 larvae about 48 hours old placed on 5 gm. flour, July 30.

431. Same as 430, except larvae were about 24 hours old. Experiment was begun July 31.

432. 10 gm. ether-extracted flour mixed with 0.2 gm. olive oil dissolved in ether. 20 larvae about 48 hours old placed on 5 gm. flour, July 30.

433. Same as 432, except larvae were about 24 hours old and experiment was started July 31.

434. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, July 30.

440. 20 larvae about 24 hours old placed on 5 gm. entire-wheat flour extracted with anhydrous ether, for 18 hours, Soxhlet, July 31. (42.3 gm. flour extracted). 1 gm. extracted flour equivalent to 3.2 c. c. ether extract.

441. Same as 440.

442. 10 gm. ether-extracted flour (see 440) mixed with 32 c. c. ether extract from same. 20 larvae about 24 hours old placed on 5 gm. flour, July 31.

443. Same as 442.

444. 10 gm. ether-extracted flour (see 440) mixed with 0.28 gm. butter dissolved in ether. 20 larvae about 24 hours old placed on 5 gm. flour, July 31.

445. Same as 444.

446. 10 gm. ether-extracted flour (see 440) mixed with 0.28 gm. lard dissolved in ether. 20 larvae about 24 hours old placed on 5 gm. flour, July 31.

447. Same as 446.

448. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour July 31.

460. 15 gm. chloroform-extracted entire-wheat flour (from a 50-gm. lot extracted 29 hours and 20 minutes with chloroform, U. S. P.; 3 changes of solvent) + 6 c. c. chloroform extract of butter oil. The butter-oil extract was prepared as follows: 50 gm. butter was dissolved in 200 c. c. absolute alcohol by heating at 50° C. for a few minutes. It was then cooled slowly to room temperature, after which the supernatant alcoholic solution was decanted from the lower less-soluble fats and insoluble material. The alcoholic solution was then evaporated nearly to dryness before a fan, at room temperature, and the residue was dried over calcium chloride for 14 days. It was then taken up in 50 c. c. chloroform U. S. P. 1 c. c. of the chloroform solution held 0.135 gm. soluble material. 20 larvae about 36 hours old placed on 5 gm. of this food, September 30.

461. Same as 460.

462. Same as 460.

463. 20 larvae about 36 hours old placed on 5 gm. of the above chloroform-extracted flour without the addition of butter oil, September 30.

464. (Control.) 20 larvae about 36 hours old placed on 5 gm. normal entire-wheat flour, September 30.

ments 358 and 359, and 59 moths from the uncounted progeny of one pair of moths in experiment 363, but in every case the growth rate was considerably retarded. Experiments 221 and 222, started with 20 larvae, each failed to produce moths. When the chloroform extract was returned to the extracted flour, growth became nearly normal. Experiments 360, 361, and 364 are in good agreement with the control, 362. Experiments 223 and 224 required a somewhat longer time for growth than the control, 225.

Flour extracted with chloroform for 24 hours at room temperature, with one change of solvent, would not permit growth of larvae to more than 3 mm. in length, and in no case did moths emerge

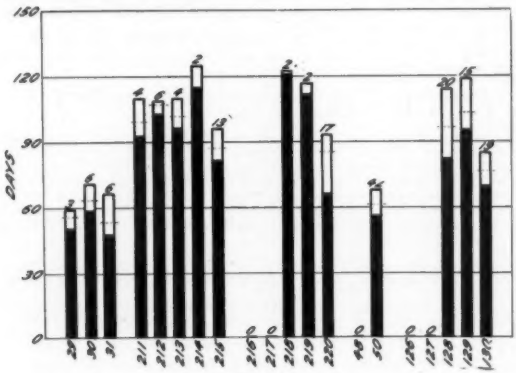


FIG. 6

48. 40 gm. entire-wheat flour extracted with acetone U. S. P., 10 hours, Soxhlet apparatus. 2 males, 2 females placed on 15 gm. flour, August 9.
50. (Control.) Entire-wheat flour. 2 males, 2 females placed on 15 gm. flour, August 9.
29. 50 gm. entire-wheat flour extracted with 200 c. c. acetone U. S. P., for 24 hours, at room temperature. 3 males, 3 females placed on 15 gm. extracted flour, July 16. (All larvae badly diseased.)
30. Duplicate of 29. (All larvae badly diseased.)
31. (Control.) Entire-wheat flour. 3 males, 3 females isolated on 15 gm. flour, July 17. (All larvae badly diseased.)
126. 39 gm. entire-wheat flour extracted with acetone U. S. P., 10 hours, Soxhlet apparatus. 20 larvae about 48 hours old placed on 5 gm. flour, December 2.
127. Duplicate of 126.
128. Half of the extracted flour from 126 mixed with half of the acetone extract, and the solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, December 2.
129. Duplicate of 128.
130. (Control.) Entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, December 2.
211. 45.8 gm. entire-wheat flour extracted 4 hours, acetone C. P., Soxhlet apparatus. 20 larvae about 24 hours old placed on 5 gm. flour, February 25.
212. Duplicate of 211.
213. Half of the extracted flour from 211 mixed with half of the acetone extract, and the solvent evaporated at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, February 25.
214. Duplicate of 213.
215. (Control.) Entire-wheat flour. 20 larvae about 24 hours old placed on 5-gm. flour, February 25.
216. 42.7 gm. entire-wheat flour extracted 7 hours, acetone C. P., Soxhlet apparatus. 20 larvae about 24 hours old placed on 5 gm. flour, February 26.
217. Duplicate of 216.
218. Half of the extracted flour from 216 mixed with half of the acetone extract, and the solvent evaporated at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, February 26.
219. Duplicate of 218.
220. (Control.) Entire-wheat flour. 20 larvae about 24 hours old placed on 5 gm. flour, February 25.

(402, 403, 411, and 412, fig. 5). When the chloroform extract was added to the residue, the larvae grew at a normal rate (404, 405, 413, and 414, fig. 5).

The use of chloroform at higher temperatures (in a Soxhlet apparatus) was attended by a marked lowering of the growth value of the treated flour. Experiments 226 and 227 (fig. 9), where the flour had been extracted for five hours, were practically identical with 228

and 229, where the residue had the extract returned to it. Both sets of experiments have a longer life cycle than the control 230, and a smaller average number of moths emerged from them. These experiments plainly indicate that an alteration or destruction of some substance occurred in the flour, and if the solvent removed any of the growth-promoting substance, the effects of the latter were nullified by the treatment. A 10-hour extraction in a Soxhlet apparatus prevented growth in three experiments (57, 58, and 235) and allowed very slow growth, with emergence of a few moths in one experiment (236). The residue when mixed with the extract (237 and 238) gave only a slightly shorter life cycle than the extracted

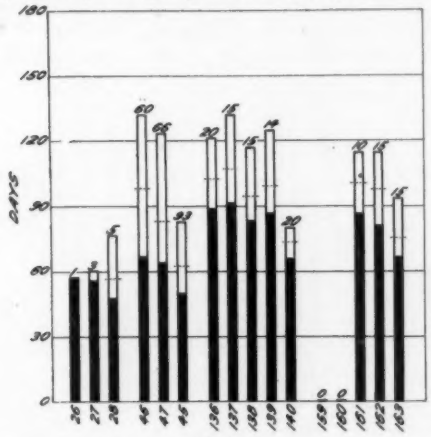


FIG. 7

45. (Control.) Entire-wheat flour. 2 males, 2 females placed on 15 gm. flour, August 4.
46. 40 gm. entire-wheat flour extracted 10 hours, benzene C. P., Soxhlet apparatus. 2 males, 2 females, placed on 15 gm. flour, August 6.
47. Duplicate of 46.
26. 50 gm. entire-wheat flour extracted with 200 c. c. benzene C. P., for 24 hours at room temperature. 3 males, 3 females on 15 gm. flour, July 9 and 10. (Larvae badly diseased.)
27. Duplicate of 26, except that experiment was started July 15. (Larvae badly diseased.)
28. (Control.) Entire-wheat flour. 3 males, 3 females placed on 15 gm. flour, July 15. (Larvae badly diseased.)
136. 41.8 gm. entire-wheat flour extracted 10 hours, benzene C. P., Soxhlet apparatus. 20 larvae about 48 hours old placed on 5 gm. flour December 13.
137. Duplicate of 136.
138. Half of the extracted flour from 136 was mixed with half of the benzene extract, and the solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. of this flour, December 13.
139. Duplicate of 138.
140. (Control.) Entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, December 13.
159. 45 gm. entire-wheat flour, extraction 15 hours benzene, Soxhlet apparatus. 20 larvae about 48 hours old placed on 5 gm. flour, December 22.
160. Duplicate of 159.
161. Half of the extracted flour from 159 mixed with half of the benzene extract, and the solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, December 22.
162. Duplicate of 161.
163. (Control.) Entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, December 22.

flour (236), again indicating a change of some kind in the flour as a result of the extraction process. The emergence record of these experiments was also very poor.[7]

[7] Experiments made with purified chloroform since the present report was written have given different results. Flour extracted for 10 hours in a Soxhlet apparatus permitted growth at about the normal rate; and when the extracted material was added to this flour, growth was also normal. The chloroform U. S. P. was purified by shaking a number of times with 5 per cent sodium thiosulphate solution, then with distilled water. It was afterwards dried over calcium chloride and distilled just before using. Some of the effects of chloroform noted above were probably the result of impurities. Such impurities, however, did not affect the flour when the same sample of chloroform was used for the extraction at room temperature.

Chloroform was the only solvent used in these experiments which approximately removed a growth-promoting substance from entire-wheat flour in 24 hours at room temperature. The substance so extracted very probably was vitamin A.

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH CARBON TETRACHLORIDE

Entire-wheat flour was extracted with carbon tetrachloride at room temperature for 24 hours, and for 10 and 15 hours in a Soxhlet apparatus, with results which are shown graphically in Figure 10. Although many of the larvae in experiments 31 to 33 were destroyed by disease, it is certain that extraction at room temperature for a 24-hour period did not remove enough of the beneficial substance to retard growth appreciably. Ten hours' extraction in a Soxhlet

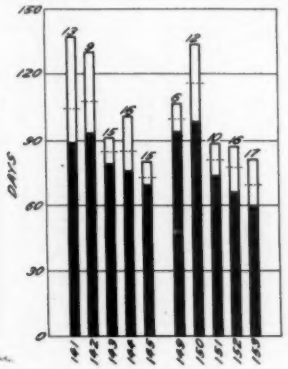


FIG. 8

141. 43.3 gm. entire-wheat flour extracted 9¾ hours with petroleum ether (b. p. 33° to 66° C.), Soxhlet apparatus. 20 larvae about 48 hours old placed on 5 gm. flour, December 13.
142. Duplicate of 141.
143. Half of the extracted flour from 142 mixed with half of the petroleum ether extract, and the solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, December 13.
144. Duplicate of 143.
145. (Control.) Entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, December 13.
149. 42.5 gm. entire-wheat flour extracted 15 hours with petroleum ether (b. p. 33° to 66°C.), Soxhlet apparatus. 20 larvae about 48 hours old placed on 5 gm. flour, December 15.
150. Duplicate of 149.
151. Half of the extracted flour from 149 mixed with half of the petroleum ether extract, and the solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, December 15.
152. Duplicate of 151.
153. (Control.) Entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, December 15.

apparatus caused a prolongation of the life cycle which was less marked in the extracted flour after the extract had been returned to it (242, 243). Flour extracted for 15 hours seemed to be still less favorable for growth, as shown by the small number of moths that reached maturity (245, 246).[8]

On the whole, carbon tetrachloride was a poor solvent for the growth-promoting substance under the conditions obtaining in these experiments.

[8] Similar results were obtained with flour which had been extracted 15 hours with purified carbon tetrachloride. The reagent was purified by shaking with concentrated sulphuric acid and allowing it to stand overnight in contact with the acid, after which it was distilled from a strong sodium hydroxide solution.

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH CARBON DISULPHIDE

Carbon disulphide did not remove an appreciable amount of the growth-promoting substance during 24 hours' extraction at room temperature (39, fig. 11). Ten hours' extraction in a Soxhlet apparatus produced a food which allowed slow growth with poor emergence

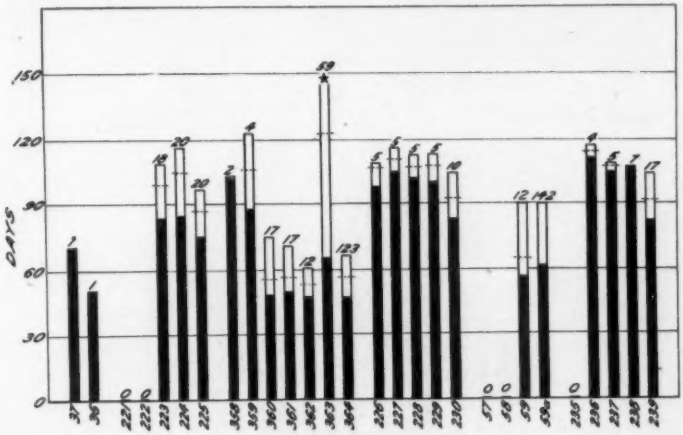


FIG. 9

57. 40 gm. entire-wheat flour extracted 10 hours, with chloroform C. P., Soxhlet. 2 fertilized females placed on 15 gm. flour, August 22. No larvae hatched from eggs laid by these females. 1 male, 1 female were placed on the same flour October 21. That larvae hatched from eggs laid by the female introduced last was evidenced by web found in the flour.

58. Duplicate of 57. Silken web was found in this flour, indicating that larvae hatched from eggs laid by the moths introduced last.

59. (Control.) 1 male, 2 females, placed on 15 gm. normal entire-wheat flour, August 22 and 23.

59a. (Control.) 1 male, 1 female, placed on 15 gm. normal entire-wheat flour, October 22.

36. (Control.) 3 males, 3 females, placed on 15 gm. normal entire-wheat flour, July 22.

37. 50 gm. entire-wheat flour extracted with 200 c.c. chloroform C. P., for 24 hours, at room temperature. 3 males, 3 females, isolated on 15 gm. flour, July 22. Larvae in 36 and 37 were very badly diseased, which accounts for the small emergence.

221. 50 gm. entire-wheat flour extracted with 200 c. c. chloroform U. S. P., for 24 hours, at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, February 26.

222. Duplicate of 221.

223. Half of the extracted flour from 221 was mixed with half of the chloroform extract, and the solvent was evaporated at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, February 26.

224. Duplicate of 223.

225. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, February 26.

226. 40.7 gm. entire-wheat flour extracted 5 hours with chloroform U. S. P., Soxhlet. 20 larvae about 48 hours old placed on 5 gm. flour, February 28.

227. Duplicate of 226.

228. Half of the extracted flour from 226 was mixed with half of the chloroform extract, and the chloroform was evaporated off at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, February 28.

229. Duplicate of 228.

230. (Control.) 20 larvae about 48 hours old isolated on 5 gm. entire-wheat flour, February 28.

235. 42.4 gm. entire-wheat flour extracted 10 hours with chloroform (purified for anesthesia), Soxhlet. 20 larvae about 48 hours old placed on 5 gm. flour, March 2.

236. Duplicate of 235.

237. Half the extracted flour from 235 mixed with half of the chloroform extract. Solvent evaporated off at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, March 2.

238. Duplicate of 237.

239. (Control.) 20 larvae about 48 hours old placed on 5 gm. entire-wheat flour, March 2.

358. 50 gm. entire-wheat flour extracted with 200 c. c. chloroform, U. S. P., for 25 hours at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, May 29.

359. Duplicate of 358.

360. Half of the extracted flour from 358 mixed with half of the extract. Solvent evaporated off at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, May 29.

361. Duplicate of 360.

362. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, May 29.

363. Larvae 24 hours old from a pair of moths placed on 14 gm. extracted flour (same as 358), May 29. *The last moth to emerge required 215 days.

364. Larvae 24 hours old from a pair of moths placed on 14 gm. extracted flour, with the extract returned (same as 360), May 29.

in two experiments (131 and 132), and no growth at all in experiments 250 and 251. After the extract was replaced in the extracted flour the life cycle was still considerably longer than that of the control in experiments 133 and 134, although emergence was much better than in the same flour without the extract. However, no growth at all occurred in 252, and only a trace of silken web was found in 253, which would indicate a little growth during the early larval stage in the latter experiment. The writer is unable to account for the lack

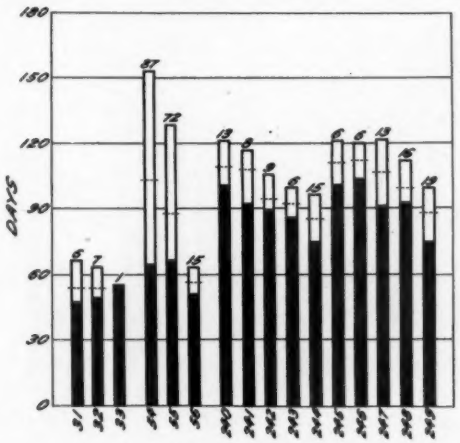


FIG. 10

31. (Control.) 3 males, 3 females, isolated on 15 gm. normal entire-wheat flour, July 17. (Larvae badly diseased.)
32. 50 gm. entire-wheat flour extracted with 200 c. c. carbon tetrachloride, C. P., for 24 hours, at room temperature. 3 males, 3 females, isolated on 15 gm. flour, July 17 and 18. (Larvae badly diseased.)
33. Duplicate of 32, July 18. (Larvae badly diseased.)
54. 40 gm. entire-wheat flour extracted for 10 hours, in a Soxhlet, with carbon tetrachloride C. P. 2 males, 2 females, isolated on 15 gm. flour, August 19.
55. Duplicate of 54.
56. (Control.) 1 male, 2 females, isolated on 15 gm. normal entire-wheat flour, August 19. The small emergence was regarded as due to disease.
240. 43 gm. entire-wheat flour extracted for 10 hours, in a Soxhlet, with carbon tetrachloride C. P. 20 larvae about 24 hours old placed on 5 gm. flour, March 3.
241. Duplicate of 240.
242. Half of the extracted flour from 240 was mixed with half of the carbon tetrachloride extract, and the solvent evaporated off at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, March 3.
243. Duplicate of 242.
244. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 3.
245. 42.2 gm. entire-wheat flour extracted 15 hours, Soxhlet, with carbon tetrachloride C. P. 20 larvae about 24 hours old placed on 5 gm. flour, March 3.
246. Duplicate of 245.
247. Half of the extracted flour from 245 was mixed with half of the carbon tetrachloride extract, and the solvent evaporated off at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, March 3.
248. Duplicate of 247.
249. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 3.

of growth in experiments 250 to 253. The carbon disulphide employed was of the same brand as that used in experiments 131 to 134, but from a different lot. No analysis of the sample used was made, but the lot from which it came was analyzed by the United States Bureau of Chemistry and found to contain no more than traces of impurities. The larvae did not grow at all in experiments 255 to 258, in which the flour was extracted for 15 hours in a Soxhlet apparatus. The carbon disulphide employed in the 15-hour extraction was from

the same container as that used for the 10-hour extraction (experiments 250 to 253).[9]

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH ETHYL ALCOHOL

Entire-wheat flour was extracted in a Soxhlet apparatus with 95 per cent alcohol for 15 hours (fig. 4). The results as shown by the length of the life cycle indicate the removal of some of the material necessary for growth, but the extraction was by no means complete

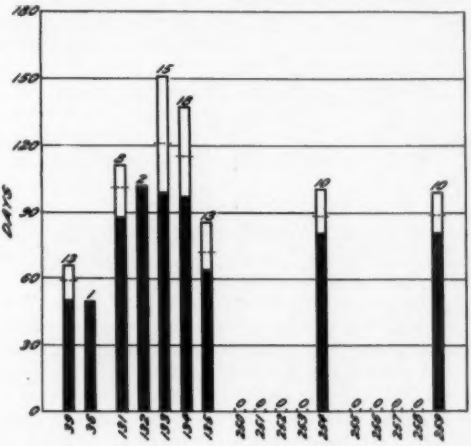


FIG. 11

36. (Control.) 3 males, 3 females, isolated on 15 gm. normal entire-wheat flour, July 22. (Diseased.)
39. 50 gm. entire-wheat flour extracted 24 hours, at room temperature, with 148 c. c. carbon disulphide C. P. 3 males, 3 females, on 15 gm. flour, July 22. (Diseased.)
131. 39.2 gm. entire-wheat flour extracted 10 hours, Soxhlet, with carbon disulphide C. P. 20 larvae about 24 hours old placed on 5 gm. flour, December 5.
132. Duplicate of 131.
133. Half of the extracted flour from 131 mixed with half of the carbon disulphide extract, and the solvent evaporated off at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, December 5.
134. Duplicate of 133.
135. (Control.) 20 larvae about 24 hours old isolated on 5 gm. normal entire-wheat flour, December 5.
250. 42.3 gm. entire-wheat flour extracted 10 hours, Soxhlet, with carbon disulphide C. P. 20 larvae about 24 hours old isolated on 5 gm. flour, March 5.
251. Duplicate of 250.
252. Half of the extracted flour from 250 mixed with half of the carbon disulphide extract, and solvent removed by evaporation at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, March 5.
253. Duplicate of 252.
254. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 5.
255. 43.4 gm. entire-wheat flour extracted 15 hours, Soxhlet, with carbon disulphide C. P. 20 larvae about 24 hours old placed on 5 gm. flour, March 6.
256. Duplicate of 255.
257. Half of the extracted flour from 255 mixed with half of the carbon disulphide extract, solvent removed by evaporation at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, March 6.
258. Duplicate of 257.
259. (Control.) 17 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 6.

(experiments F–26 and F–27). The emergence also was poor in comparison to that in the control (F–30). However, the extracted flour plus the alcoholic extract showed a nearly normal growth rate (F–37

[9] Since the above was written, data have been obtained for flour extracted with purified carbon disulphide for 15 hours in a Soxhlet apparatus. Only one of 40 larvae was alive when the bottles were examined 82 days after the experiments started. The extracted flour plus extract, however, was apparently normal in its growth-promoting ability. The purified reagent was prepared from C. P. carbon disulphide, which was held in contact with anhydrous copper sulphate for two days with occasional shaking and distilled from anhydrous copper sulphate just before using. The writer is inclined to believe that the results noted in experiments 250 to 253, and 255 to 258, resulted, in part at least, from impurities, probably decomposition products, in the solvent used.

and F–38).[10] In another experiment, begun on June 19, 10 larvae about half grown (5 to 7 mm. in length) were placed on 10 gm. of the extracted flour from the same lot as that used in F–26 and F–27. The first moth to appear required 33 days for growth and transformation; or about one-half the time required by the first moth to emerge in experiment F–27, which was 68 days. Six other moths emerged later in this experiment.

The relation of the growth value of a 95 per cent alcoholic extract from entire-wheat flour (10 hours, Soxhlet) to that of an ether extract (15 hours, Soxhlet) is shown in Figure 4. In flour from which first the ether-soluble and then the 95 per cent alcohol-soluble materials were removed and the alcohol-soluble substances then replaced (118 and 119), growth continued slowly for a long time and a single moth emerged from 119 after 275 days. When the ether-soluble material only was replaced, the life cycle was prolonged, but a good emergence of moths was obtained (120 and 121). With both the alcohol-extracted and ether-extracted materials replaced, the rate of growth was still better (122 and 123). It is evident from this series of experiments that under these conditions the ether extracted more of an important growth-stimulating factor than did the alcohol.

Two series of experiments were made with entire-wheat flour extracted for 10 and 15 hours, respectively, with absolute (99+per cent) alcohol in a Soxhlet apparatus. The results are given in Figure 4 (170–179). Extraction of the growth-promoting substance was not complete in either case, and the extracted flour with the extracted material replaced was even less efficient than the extracted flour itself. It is clear that some constituent of the extracted material was changed in such a way as to check the growth of the larvae.

Cold extraction (room temperature) with 95 per cent alcohol for 24 hours resulted in an incomplete removal of the growth-promoting substance (435 and 436), and growth was still slow after the extracted material was replaced in the extracted flour (437 and 438). Thus, even at room temperature, alcohol had a harmful action on the flour.

GROWTH IN EXTRACTED ENTIRE-WHEAT FLOUR SUPPLEMENTED WITH FAT-SOLUBLE VITAMIN AND FATS

In order to gain an insight into the nature of the growth-promoting substance removed from entire-wheat flour by means of ether and chloroform, extracted flour was supplemented with substances rich in vitamin A, as well as substances consisting largely of fats which at most contain only small quantities of this vitamin. Butter or ether extract of egg yolk supplied vitamin A, and lard or olive oil furnished the fats, the additions forming approximately 2 per cent or more of the diet. A portion of each lot of extracted flour with the extract from it replaced was tested also to determine whether its ability to promote growth had been affected by the extraction process.

The results of these experiments are shown in Figure 5. The flour extracted at room temperature with chloroform did not permit growth to maturity (402, 403, 411, and 412), whereas the same

[10] This experiment has recently been repeated with flour extracted 15 hours in a Soxhlet apparatus with 96 per cent alcohol. The results with the extracted flour alone agree with those given above, but with the extracted flour plus extract growth was retarded as observed in the experiments with abolute alcohol. The purified alcohol was prepared from the commercial 95 per cent grade by treatment with solid sodium hydroxide for six hours, distilling and digesting the distillate with m-phenylenediamine for six days, then distilling again just before using.

flour with the chloroform extract returned to it permitted growth and transformation in practically normal time (404, 405, 413, and 414), as shown by comparison with the controls (410 and 415). The egg-yolk extract (406 and 407) rendered the extracted flour the equal of the normal untreated flour in every way. On the other hand, when butter was added to the extracted flour growth did not result, (416 and 417) and in this respect it gave the same results as olive oil (408 and 409) and lard (418 and 419).

Comparable results were obtained with the flour which was extracted with ether for 18 hours in a Soxhlet apparatus. The extracted flour would not support growth (426, 427, 440, and 441) until the extract was added to it (442 and 443), after which the growth rate was practically normal. The ether-extracted substance from egg yolk when added to the extracted flour promoted growth at a rate comparable to that in normal entire-wheat flour (428 to 431). The addition of 5.7 per cent of ether-soluble substance from egg yolk was no more effective than 2 per cent. Again butter was not effective in supporting growth (444 and 445), but gave the same negative results as olive oil (432 and 433) and lard (446 and 447).

The results obtained with butter were rather surprising, in view of the fact that it has been repeatedly used in experiments with rats as the sole source of vitamin A. That butter contains a substance detrimental to the growth of Ephestia larvae seems doubtful from the results obtained with highly milled flour supplemented with butter (fig. 13, 272, 273, 274.) Here, the life cycle was no longer than in the untreated highly milled flour. A possible explanation is that the fat-soluble vitamin in the butterfat gradually deteriorated during the course of the experiments, since freshly prepared food was not supplied after the experiments were started.[11] But the negligible amount of growth as shown by the small quantity of silken web in the flour suggests that there was not enough vitamin present at the beginning of the experiment to sustain growth for any length of time.

In order to determine whether butter contained a growth-promoting substance which would supplement the chloroform-extracted flour, a more concentrated butter preparation was sought. Butter oil, i. e., the portion soluble in cold absolute alcohol, was prepared with some modifications after the method of Osborne and Wakeman (*27*). It was added to the extracted flour at a concentration of 5.1 per cent of the resulting mixture. The results given in Figure 5 (460 to 462) indicate a satisfactory emergence of moths and a growth rate which approached but was still slower than that of the control (464). The extracted flour without butter oil did not support growth at all (463). Osborne and Mendel (*28*) observed that the growth-promoting factors were more concentrated in the butter oil than in the butter fat. These experiments agree with theirs in this respect.

The conclusion is reached from these experiments that a substance is present in the ether-extract of egg yolk which meets all the requirements of growth and the production of normal-sized moths when added to extracted entire-wheat flour that will not otherwise sustain life. It appears to be in every way as efficient as the ether and chloroform extracts of entire-wheat flour. Olive oil and lard do not

[11] Hopkins (*18*) and Drummond and Coward (*11*) have observed a considerable reduction of fat-soluble vitamin in butterfat which has been exposed to the air at ordinary temperatures.

contain this substance, or if it is present it exists in such small quantities or in such a form that it is not available for the larvae.

Butterfat also contains a substance which has a beneficial influence on growth, but it is present in a considerably lower concentration than in the egg-yolk extract. It can be obtained in a more concentracted condition in a suitable butter-oil preparation. The active substance in egg yolk and butterfat quite probably is vitamin A.

GROWTH IN HIGHLY MILLED (PATENT) WHEAT FLOUR

Early in the course of these experiments it was observed that the growth of Ephestia larvae was slower in highly milled flour than in normal entire-wheat flour. In Figure 2 the length of the life cycle of the first moth to emerge in highly milled flour (upper curve) is compared with the same life cycle in normal entire-wheat flour (lower curve). In 18 experiments with highly milled flour the first moth emerged in 110 days or more in all but four cases; the controls did not exceed 70 days in 13 experiments; and if data had been available for February they undoubtedly would have shown that not more than 85 days were required for the first emergence, a developmental time only occasionally approached by larvae grown in highly milled flour. Additional data on the growth in highly milled flour are given in Figures 12 and 13.

Not only is the baneful effect of highly milled flour shown in slow growth but the moths which reach maturity are considerably smaller than those fed normal entire-wheat flour. Sixteen males selected at random gave a mean weight of 0.0085 gm. and 13 females a mean weight of 0.0103 gm. A comparison of these figures with the weights of moths from normal flour given in Table I reveals the fact that the males from highly milled flour attain only about 60 per cent and the females about 50 per cent of the weight of the respective sexes from normal flour.

Chittenden (*9*) compared the life cycle of Ephestia larvae in highly milled flour with that in yellow-maize meal. In the former 114 days were required, in the latter 52 days. These figures are approximately the same as have been obtained in this investigation with highly milled flour and entire-wheat flour.

In regard to the food of the larvae Burkhardt (*5*) makes the following significant remarks: "Besondere Vorliebe vor allen anderen Futterstoffen zeigt die Larve für Griess, sei es von Mais, Weizen oder anderem Getreide, wie denn überhaupt die groben Vermahlungen mehr geschätzt werden als die feinen Mahlprodukte, wie beispielsweise das sog. feine Auszugmehl." The coarser wheat milling products carry most of the embryo. Its significance for growth will be discussed under the next heading.

Highly milled flour probably will not affect the growth of all grain-infesting insects in like degree. Chapman (*6*) shows that the life cycle of the confused flour beetle (*Tribolium confusum* Duval) is slightly longer in some cereal foods than in others, but apparently finds much less difference than is evident in the growth response of Ephestia.

GROWTH IN FLOUR FROM DEGERMINATED WHEAT

Highly milled wheat flour consists very largely of wheat endosperm with a small amount of bran and possibly traces of embryo present as impurities. It was thought that the relatively slow growth made

by Ephestia larvae in this flour might have been the result either of the low concentration of the bran or embryo constituents, or possibly of the paucity of both these ingredients, which are present in comparatively large amounts in the entire-wheat flour. Since only a small amount of flour was required, the embryos were removed by cutting off the tip of each kernel which contained the embryo. The wheat thus degerminated was ground in a mortar to a coarse flour that contained more than half of the branny coats of the original kernels.

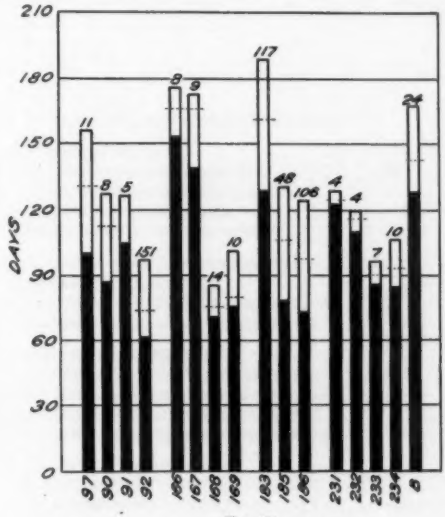


FIG. 12

90. 1 male, 1 female, placed on 15 gm. highly milled (patent) flour, October 6. (Badly diseased.)
91. Duplicate of 90. (Badly diseased.)
92. (Control.) 1 male, 1 female, placed on 15 gm. normal entire-wheat flour, October 6.
97. 1 male, 1 female, placed on 1 gm. flour from degerminated wheat, October 9. One gm. degerminated flour added on November 3 and on November 10. (Badly diseased.)
166. 20 larvae about 72 hours old placed on 5 gm. highly milled flour, December 26.
167. Duplicate of 166.
168. (Control.) 20 larvae about 72 hours old placed on 5 gm. normal entire-wheat flour, December 26.
169. Duplicate of 168.
183. 1 male, 1 female, placed on 16.3 gm. flour from degerminated wheat, February 9.
185. (Control.) 1 male, 1 female, placed on 14.1 gm. normal entire-wheat flour, February 9.
186. Duplicate of 185.
231. 20 larvae about 48 hours old placed on 2 gm. flour from degerminated wheat, March 1.
232. Duplicate of 231.
233. (Control.) 20 larvae about 48 hours old placed on 2 gm. normal entire-wheat flour, March 1.
234. Duplicate of 233.
B. Average of experiments 262, 263, 264, and 265 (fig. 13). 20 larvae about 48 hours old placed on 5 gm. highly milled flour, March 11, in each experiment.

The growth rate in the degerminated flour is shown in Figure 12, where comparison can be made with both the highly milled and the normal entire-wheat flours. Although the larvae in experiment 97 were badly diseased, the growth rate is similar to that in 90 and 91—both of which were also badly diseased—where highly milled flour was used. It is certainly much slower than the rate in normal flour (92). Experiment 183, in which no diseased larvae were observed, produced a good number of moths, but the life cycle was long, characteristic of that observed in highly milled flour when growth occurred largely during the cold part of the year (166 and 167). Experiments 231 and 232, begun on March 1, have a shorter life

cycle, since the greater part of the growth occurred during the spring. They are comparable to experiments 262 to 265, Figure 13,

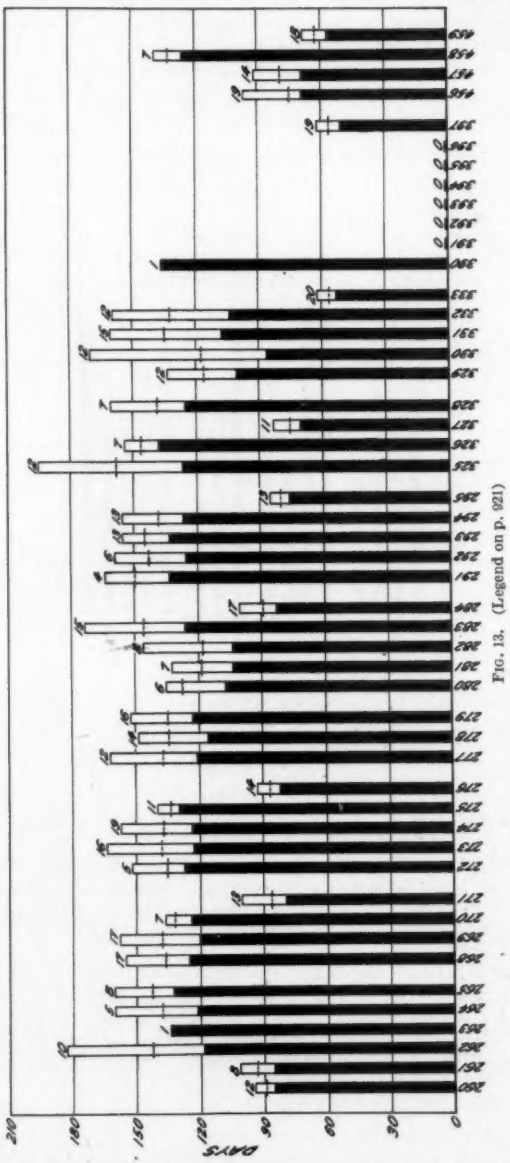


FIG. 13. (Legend on p. 921)

where the food consisted entirely of highly milled flour. These latter four experiments are averaged and shown in B in Figure 12.

EXPLANATORY LEGEND FOR FIG. 13

260. (Control.) 20 larvae about 48 hours old placed on 5 gm. entire-wheat flour, March 11.
261. Duplicate of 260.
262. 20 larvae about 48 hours old placed on 5 gm. highly milled flour, March 11.
263. Same as 262.
264. Same as 262.
265. Same as 262.
268. 16 gm. highly milled flour mixed with 30 c. c. ether extract from 45.2 gm. entire-wheat flour (15 hours, Soxhlet.); ether evaporated off at room temperature. 1 c. c. extract equivalent to 0.628 gm. entire-wheat flour. 20 larvae about 24 hours old placed on 5 gm. flour, March 16.
269. Same as 268.
270. Same as 268.
271. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 16.
272. 50 gm. highly milled flour mixed with 1 gm. butter dissolved in ether; solvent removed by evaporation at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, March 19.
273. Same as 272.
274. Same as 272.
275. 20 larvae about 48 hours old placed on 5 gm. highly milled flour, March 19.
276. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, March 19.
277. 50 gm. highly milled flour mixed with 1 gm. olive oil dissolved in ether; solvent removed by evaporation at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, March 19.
278. Same as 277.
279. Same as 277, except that 21 larvae were used.
280. 25 gm. highly milled flour mixed with 25 c. c. of a 50 per cent alcoholic extract of yeast. Alcohol removed by evaporation at room temperature. Yeast extract was prepared as follows: 69.3 gm. moist cakes of bakers' yeast crushed in a mortar, 30 c. c. ether being added to kill the cells. 230 c. c. of 50 per cent alcohol added gradually, after which the mixture was transferred to a flask and allowed to stand 24 hours with occasional shaking. Mixture filtered, first through cloth and then through folded paper filter. 1 c. c. of the recovered extract contained 0.0139 gm. solids. 20 larvae about 24 hours old placed on 5 gm. flour, March 20.
281. Same as 280.
282. Same as 280.
283. 20 larvae about 24 hours old placed on 5 gm. highly milled flour, March 20.
284. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 20.
291. 50 gm. highly milled flour mixed with 1 gm. lard dissolved in ether. Solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, March 22.
292. Same as 291.
293. Same as 291.
294. 20 larvae about 48 hours old placed on 5 gm. highly milled flour, March 22.
295. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, March 22.
325. 8 gm. highly milled flour mixed with 2 gm. purified casein. Purified casein was prepared from commercial casein by dissolving in weak sodium hydroxide solution, precipitating with acetic acid, washing free of acid with distilled water by decantation, then extracting three times with hot absolute alcohol and two times with anhydrous ether. It was then dried and ground. 20 larvae about 48 hours old placed on 5 gm. mixture, April 26.
326. Same as 325.
327. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, April 26.
328. 20 larvae about 48 hours old placed on 5 gm. highly milled flour, April 26.
329. 10 gm. highly milled flour mixed with 0.2 gm. salt mixture. Composition of salt mixture: 0.1 gm. $CaCO_3$; 1.0 gm. $MgSO_4.7H_2O$; 0.5 gm KCl; 3 gm. $Na_2HPO_4.12H_2O$. 20 larvae about 24 hours old placed on 5 gm. flour plus salt mixture, May 20.
330. Same as 329.
331. 10 gm. highly milled flour mixed with 0.2 gm. salt mixture (see 329 for composition) and 20 c. c. of an ether extract of egg yolk. Preparation of egg-yolk extract: 5 gm. air-dried egg yolk finely chopped and extracted with six successive 30 c. c. portions of anhydrous ether, each portion being heated to incipient boiling. 1 c. c. of the resulting filtered ether extract contained 0.02 gm. solids. 20 larvae about 24 hours old placed on 5 gm. of this mixture, May 20.
332. Same as 331.
333. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, May 20.
390. 20 larvae about 48 hours old placed on 5 gm. highly milled flour, June 21. (Discarded by mistake after first moth emerged.)
391. 15 gm. highly milled flour mixed with 147 c. c. of a 95 per cent alcoholic extract of entire-wheat flour. Solvent removed before fan at room temperature. Extract was prepared from 30 gm. entire-wheat flour given four extractions with boiling 95 per cent alcohol, within 7 hours. 147 c. c. of extract was equivalent to 15 gm. original entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, June 21.
392. Same as 391.
393. Same as 391.
394. 15 gm. highly milled flour mixed with 147 c. c. 95 per cent alcoholic extract of entire-wheat flour (see 391) and 13 c. c. of an ether extract of entire-wheat flour. Ether extract prepared from 47.7 gm. entire-wheat flour treated for 15 hours, in Soxhlet. 1 c. c. equivalent to 0.85 gm. original entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, June 21.
395. Same as 394.
396. Same as 394.
397. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, June 21.
456. 25 gm. highly milled flour + 230 c. c. of a 50 per cent alcoholic extract of yeast. Evaporated to dryness before a fan at room temperature. Yeast extract prepared as follows: 71.5 gm. moist bakers' yeast was stirred into 150 c. c. boiling distilled water. Mixture was cooled, 150 c. c. absolute alcohol was added, and mixture allowed to stand 24 hours. 1 c. c. of the filtered extract contained 0.0086 gm. solids. 20 larvae 48 hours old placed on 5 gm. of this food, September 18.
457. Same as 456.
458. 20 larvae 48 hours old placed on 5 gm. highly milled flour, September 18.
459. (Control.) 20 larvae 48 hours old placed on 5 gm. normal entire-wheat flour, September 18.

Experiments 231 to 234 are interesting in another connection. Although the usual number of larvae (20) was employed, only 40 per cent of the food generally allowed was given them. The emergence was poor in both the degerminated and normal flours, but fewer than half as many moths emerged in the degerminated flour as in the latter. The facts point to the necessity for a larger amount of degerminated flour in order to complete growth and transformation, but further data will be required to substantiate them.

The weight of the newly-emerged moths from degerminated flour is apparently only a little below the average for moths from normal flour, being 0.0130 gm. for the single male weighed, and an average of 0.0200 gm. for seven females. (See Table I.)

On the basis of these experiments, it is evident that the growth rate in flour made from wheat kernels which have had the embryos removed is about the same as the rate in highly milled wheat flour. The outstanding difference seems to be the greater weight of the moths that emerge from the degerminated flour. Wheat bran carries a higher percentage of inorganic constituents than any other part of the kernel (*32*, *p. 427*), and one might infer that the greater gain in weight made in the degerminated flour is due to the relatively higher inorganic content of its included bran. Such a conclusion does not seem justified from the results obtained with highly milled flour supplemented with a salt mixture containing most of the inorganic constituents found in the wheat kernel. (See fig. 13.) It is possible, of course, that this salt mixture lacked some essential compound or failed to present the inorganic constituents in their proper proportions.

A substance or a group of substances present in the wheat embryo is undoubtedly responsible for the difference in the growth rate of Ephestia larvae in entire wheat and degerminated wheat flours. Osborne and Mendel (*29*) found the crude protein of wheat embryo superior to the proteins of wheat endosperm for the growth and maintenance of rats. They also have given figures which show that the embryo constitutes only 1.5 per cent of the entire kernel, and contains but 4.4 per cent of the total protein of the kernel. The results of experiments given below show quite conclusively that highly milled flour, supplemented with casein, which is known to be adequate for the growth of mammals, did not hasten the growth of Ephestia larvae. But for an almost total ignorance of the protein requirements of insects, one might conclude from the above data that the growth-promoting properties of wheat embryo were due to something besides its protein content.

Voegtlin and Myers (*33*) conclude from experiments on adult pigeons that the antineuritic vitamin is located entirely in the embyro of wheat and maize. In the case of Ephestia larvae on an exclusive diet of degerminated wheat, growth can certainly take place and apparently normal moths emerge. This difference might be explained on the basis of a quantitatively smaller amount of antineuritic vitamin required for the growth and transformation of Ephestia. If this is true, the vitamin must be derived from the peripheral layers or the endosperm of the wheat kernel, or both.

The writer has not tested the growth rate on wheat bran, but from the observations of Burkhardt (*5*) it is probable that the larvae

require a longer time for growth on this food, and transform into smaller moths which lay fewer eggs, than larvae reared in coarsely ground wheat meal (Griess).

GROWTH IN HIGHLY MILLED FLOUR SUPPLEMENTED WITH VARIOUS FOOD SUBSTANCES

From the standpoint of vertebrate nutrition, the vitamin deficiency of highly milled flour is threefold. Vitamin A is entirely lacking or reduced to a very small amount, whereas vitamin B is entirely (*33*) or largely (*8, 29, 4*) absent when the embyro is removed. Apparently the antiscorbutic vitamin is present neither in highly milled flour, nor in the entire dried grain.

Aside from its greatly reduced vitamin content, highly milled flour has lost about one-half of the fat present in entire-wheat flour, and is also deficient in mineral salts. The entire wheat kernel contains 2.18 per cent fat, and wheat endosperm contains only 1.15 per cent, bran contains 8.26 per cent, and the embryo 13.51 per cent (*29*). The ash constituents of entire wheat equal about 1.37 per cent of the edible portion of the kernel, but with the removal of the bran and embryo, which together make up only 16.5 per cent of the kernel, the remaining flour has the reduced percentage of about 0.56, or less than half the amount contained in the entire wheat (*32, pp. 423, 427*).

An attempt has been made to modify highly milled flour by the addition of various substances, which, it was thought, would correct these deficiencies. An ether extract of entire-wheat flour was added to a portion of highly milled flour to increase the vitamin A content; other lots were supplemented respectively with ether extract of egg yolk and butterfat. If the deficiency were in the nature of a fat, these substances should tend to correct it. Lard and olive oil were added to portions of highly milled flour to increase the fat content. Although they may contain some vitamin A (*10*) the amount is probably small. Vitamin B was added in the form of an alcoholic extract of bakers' yeast and an alcoholic extract of entire-wheat flour. Casein was chosen as the protein to correct any deficiency in that direction. Finally, a salt mixture was added to a portion of the flour to determine whether the deficiency in growth was due to a low concentration of any of the more abundant mineral constituents of wheat.

The results of these experiments are shown in Figure 13. The fatty substances, olive oil, lard, butter, and the ether extracts of entire-wheat flour and egg yolk, when added to highly milled flour, failed to produce a significant shortening of the life cycle of Ephestia. The olive oil, lard, and butter were used at approximately 2 per cent concentrations, the egg-yolk extract at about 4 per cent of ether-soluble substance. One gm. of the highly milled flour was used for each 1.87 c. c. of ether extract of entire-wheat flour (equivalent to 1.17 gm. original flour). There can be little doubt that the failure of Ephestia larvae to grow in this flour was due neither to a deficiency in fats nor to a deficiency in vitamin A. Some fats are already present in the milled flour, and certainly one may assume that some vitamin A is present there also. The additions to milled flour as noted above were large enough to have shown a notable increase in the growth rate if fats or vitamin A were the lacking constituents.

The weight of the moths emerging from these experiments was, like the length of the life cycle, practically identical with the weight of those from untreated highly milled flour. (See Table I.)

Neither the addition of casein nor of a salt mixture improved the growth value of highly milled flour, as both the growth rate and the weight of adult moths show (fig. 13 and Table I).

Larvae grew slowly and finally died in milled flour supplemented with the material extracted from entire-wheat flour by hot 95 per cent alcohol (391, 392, 393). Other experiments in which both an alcoholic and an ether extract of entire-wheat flour were added gave no better results (394, 395, 396). Only 3 small larvae (2.5 mm. in length) out of 120 larvae remained alive at the end of 91 days in these experimental bottles. The experiments were then discontinued.

Highly milled flour to which a 50 per cent alcoholic extract of bakers' yeast was added shortened the life cycle to a value lying between that obtained in untreated milled and normal entire-wheat flour (280, 281, and 282, fig. 13). The total solids of the yeast extract equaled 1.39 gm. in 100 c. c. The treated air-dried flour contained 1.4 per cent of the yeast solids.

The above experiment was repeated with flour in which the total solids from the alcoholic yeast extract formed 7.3 per cent of the resulting mixture. The period of growth was shorter in these experiments (456 and 457, fig. 13) than in those mentioned above, but the growth rate in relation to the control was the same. The weight of the moths was similar, the mean for eight males being 0.0131 gm. and for eight females, 0.0170 gm. (compare with Table I).

Thus it is seen that an alcoholic extract of yeast, the classical source of vitamin B, was the only substance of a number tried which improved the growth value of highly milled flour. The amount of yeast substance added to the flour, especially in the first series of experiments, was so small as to suggest strongly that the ordinary food constituents of the extract were not responsible for the improvement in growth, but that the vitamin B was.

It should be noted, however, that the life cycle in this flour was still considerably longer than in entire-wheat flour. This may have been due to an insufficient amount of vitamin B, or to a deficiency of other ingredients. It is also possible that the alcohol of the yeast extract exerted a deleterious effect upon the flour. However, in view of these results and the experience of others with higher animals, the tentative conclusion is reached that the slow growth of Ephestia larvae and the abnormal weight of the moths are due, in part at least, to the low concentration of vitamin B in highly milled wheat flour.

CONCLUSIONS

Ephestia kuehniella larvae grow normally and produce normal-sized moths in flour made from the entire wheat kernel.

In entire-wheat flour, extracted at room temperature with chloroform for a 24-hour period, only a few larvae developed into moths. Extraction under the same conditions for 24 to 28 hours, but with one or more changes of solvent, removed the growth-promoting substance so completely that the larvae died at an early age. When the material extracted with chloroform was replaced in the flour, larvae grew at nearly the normal rate.

Entire-wheat flour extracted at room temperature for a 24-hour period with ether, acetone, benzene, petroleum ether (containing fractions boiling higher than 66° C.), carbon tetrachloride, or carbon bisulphide, did not prevent or greatly prolong the period of growth of the larvae. Under the same conditions, 95 per cent alcohol extracted some of the growth-promoting substance, but in the extracted flour to which the extract was added, growth did not take place at the normal rate.

TABLE 1.—*Weight of Ephestia kuehniella moths*

Kind of flour	Males	Females
	Gm.	*Gm.*
Normal entire-wheat flour	0.0113	0.0191
	.0119	.0195
	.0124	.0196
	.0125	.0197
	.0126	.0203
	.0131	.0204
	.0132	.0205
	.0137	.0206
	.0138	.0209
	.0139	.0222
	.0140	.0224
	.0140	.0225
	.0146	.0234
	.0146	.0247
	.0146	----------
	.0149	----------
	.0151	----------
	.0154	----------
	.0159	----------
Mean weight	.0138	.0211
Highly milled flour	.0061	.0066
	.0071	.0072
	.0079	.0082
	.0081	.0097
	.0084	.0099
	.0084	.0100
	.0085	.0104
	.0085	.0106
	.0086	.0107
	.0087	.0115
	.0088	.0125
	.0089	.0126
	.0091	.0138
	.0092	----------
	.0095	----------
	.0097	----------
Mean weight	.0085	.0103
Degerminated wheat flour	.0130	.0169
	----------	.0177
	----------	.0178
	----------	.0190
	----------	.0214
	----------	.0230
	----------	.0241
Mean weight	.0130	.0200
Highly milled flour, and ether extract of entire-wheat flour	.0075	.0103
	.0078	.0111
	.0085	.0112
	.0086	.0114
	.0088	.0123
	.0094	----------
	.0099	----------
	.0105	----------
Mean weight	.0089	.0113
Highly milled flour and butter	.0068	.0087
	.0071	.0093
	.0076	.0093
	.0078	.0108
	.0079	.0109
Highly milled flour and butter	0.0082	0.0110
	.0087	.0112
	.0089	----------
	.0095	----------
	.0100	----------
	.0103	----------
	.0104	----------
	.0105	----------
Mean weight	.0087	.0102
Highly milled flour, and olive oil	.0073	.0088
	.0078	.0100
	.0078	.0110
	.0079	.0110
	.0083	.0114
	.0084	.0121
	.0109	.0125
	----------	.0131
	----------	.0145
Mean weight	.0083	.0116
Highly milled flour, and alcoholic extract of yeast	.0129	.0159
	.0129	.0176
	.0134	.0191
	.0137	.0216
	.0143	----------
Mean weight	.0134	.0185
Highly milled flour, and lard	.0070	.0098
	.0076	.0101
	.0082	.0114
	.0095	----------
Mean weight	.0081	.0104
Highly milled flour, and casein	.0056	.0089
	.0062	.0093
	.0079	.0106
	.0088	.0128
	.0091	----------
	.0098	----------
Mean weight	.0079	.0104
Highly milled flour, and salt mixture	.0073	.0114
	.0076	.0115
	.0088	.0116
	.0092	.0132
	.0096	.0141
	.0129	.0147
Mean weight	.0092	.0127
Highly milled flour, mixed with ether extract of egg yolk and salt mixture	.0067	----------
	.0081	----------
	.0084	.0087
	.0087	.0117
	.0087	.0144
	.0091	.0147
	.0093	----------
	.0099	----------
	.0103	----------
Mean weight	.0088	.0124

Entire-wheat flour extracted with anhydrous ether for 15 hours or more in a Soxhlet apparatus sometimes permitted slow growth, but the larvae nearly always failed to reach the pupal stage. The extracted flour with the ether-extracted material returned to it was almost equal to the untreated flour in ability to promote growth.

Entire-wheat flour extracted in a Soxhlet apparatus with a number of other solvents gave the following results: A 10-hour treatment with purified acetone was insufficient to remove all the growth-promoting substance. Extraction with purified benzene for 15 hours also was insufficient. Neither petroleum ether (b. p. 33° to 66° C.) nor carbon tetrachloride completely extracted the growth-promoting substance in 15 hours. Extraction for 10 hours with purified chloroform permitted growth at about the normal rate. Purified carbon disulphide removed enough growth-promoting material in a 15-hour extraction to prevent moths from emerging. Ninety-five per cent and absolute ethyl alcohol affected the flour in such a way that growth was retarded after 15 hours' extraction, but moths could still emerge from it. When the 95 per cent alcoholic extract was replaced in the extracted flour, the food was nearly normal in two experiments. However, replacement of the material extracted with absolute alcohol resulted in slower growth than in the extracted flour.

The experiments indicate that certain solvents of standard grade, when used in a Soxhlet apparatus, may contain impurities which will affect the growth value of the extracted flour.

An ether extract of egg yolk when added to entire-wheat flour extracted for 18 hours in a Soxhlet apparatus with anhydrous ether permitted the growth of larvae at the normal rate.

Entire-wheat flour extracted with chloroform at room temperature for 28 hours with one change of solvent, and supplemented with an ether extract of egg yolk, promoted normal growth of Ephestia larvae.

Entire-wheat flour extracted with anhydrous ether for 18 hours in a Soxhlet apparatus, or with chloroform at room temperature for 24 hours, with several changes of solvent, and then supplemented with the ether-soluble part of butter (2.7 to 4.8 per cent), did not permit growth.

Entire-wheat flour when thoroughly extracted with chloroform at room temperature and supplemented with butter oil (i. e., the part of butterfat which is soluble in cold absolute alcohol) permitted growth at a fairly rapid but still subnormal rate.

Entire-wheat flour extracted with ether or chloroform, and supplemented with lard or olive oil, was inadequate for growth.

Highly milled (patent) wheat flour caused a retardation in the growth rate and the production of moths weighing from approximately 50 to 60 per cent of the normally fed moths.

Flour made from wheat kernels with the embryos removed, but containing more than half of the branny substance of the original seed, promoted growth at approximately the same rate as the highly milled flour. The moths which emerged from it, however, were of nearly the same weight as those maturing in normal entire-wheat flour.

Highly milled flour supplemented with olive oil, lard, butter, and ether extracts of egg yolk and of entire-wheat flour, showed no improvement in the rate of growth or the weight of the moths over

the untreated highly milled flour. When supplemented with a hot 95 per cent alcoholic extract of entire-wheat flour, moths did not emerge from the flour. The addition of an alcoholic and an ether extract of entire-wheat flour led to the same result.

Highly milled flour supplemented with purified casein, or a salt mixture containing most of the mineral constituents of wheat, did not permit more rapid growth than the highly milled flour itself.

Highly milled flour containing 1.4 per cent solids from a 50 per cent alcoholic extract of bakers' yeast, increased the growth rate and the weight of the moths to values lying between those for entire-wheat flour and highly milled flour. When the flour contained 7.3 per cent of yeast solids, the growth rate and weight of the moths were about the same as observed in the flour with the smaller content of yeast solids.

It is believed that ether and chloroform both remove a substance or substances from entire-wheat flour which are similar to, or identical with, vitamin A as understood in mammalian nutrition.

Egg yolk and butter contain a substance which promotes the growth of Ephestia larvae. It is quite probably vitamin A. It seems to be more abundant or more stable in egg yolk than in butterfat.

Since the growth value of highly milled wheat flour was considerably improved by the addition of an alcoholic extract of yeast, it is believed that the deficiency of flour for growth lies, at least partly, in its low concentration of vitamin B.

The quantity of vitamin B necessary for the growth of Ephestia at a normal rate appears to be contained largely, but not entirely, in the embryo of the wheat kernel.

LITERATURE CITED

(1) Aeppler, C. W.
1922. Tremendous growth force. Gleanings Bee Cult. 50: 151–153, illus.

(2) Bacot, A. W., and Harden, A.
1922. Vitamin requirements of drosophila. I. Vitamins B and C. Biochem. Jour. 16: 148–152.

(3) Baumberger, J. P.
1919. A nutritional study of insects, with special reference to microörganisms and their substrata. Jour. Expt. Zool. 28: 1–81, illus.

(4) Bell, M., and Mendel, L. B.
1922. The distribution of vitamin B in the wheat kernel. Amer. Jour. Physiol. 62: 145–161, illus.

(5) Burkhardt, F.
1919. Zur biologie der mehlmotte (ephestia kuehniella zeller) Ztschr. Angew. Ent. 6: 25–60, illus.

(6) Chapman, R. N.
1918. The confused flour beetle (tribolium confusum duval). Minn. State Ent. Rpt. 17: 73–94, illus.

(7) ———
1924. Nutritional studies on the confused flour beetle, tribolium confusum duval. Jour. Gen. Physiol. 6: 565–585, illus.

(8) Chick, H., and Hume, E. M.
1917. The distribution in wheat, rice, and maize grains of the substance, the deficiency of which in a diet causes polyneuritis in birds and beri-beri in man. Roy. Soc. [London] Proc. (B) 90: 44–60.

95901—26†——3

(9) CHITTENDEN, F. H.
1896. THE DEVELOPMENT OF THE MEDITERRANEAN FLOUR MOTH. U. S. Dept. Agr., Div. Ent. Bul. (n. s.) 6: 85–88.
(10) DANIELS, A. L., and LOUGHLIN, R.
1920. NOTE ON THE FAT-SOLUBLE GROWTH-PROMOTING SUBSTANCE IN LARD AND COTTON-SEED OIL. Jour. Biol. Chem. 42: 359–362, illus.
(11) DRUMMOND, J. C., and COWARD, K. H.
1920. RESEARCHES ON THE FAT-SOLUBLE ACCESSORY FACTOR (VITAMIN A). VI: EFFECT OF HEAT AND OXYGEN ON THE NUTRITIVE VALUE OF BUTTER. Biochem. Jour. 14: 734–739, illus.
(12) FUNK, C.
1922. THE VITAMINES. Translated from 2d German ed. by H. E. Dubin. 502 p., illus. Baltimore.
(13) GLASER, R. W.
1923. THE EFFECT OF FOOD ON LONGEVITY AND REPRODUCTION IN FLIES. Jour. Expt. Zool. 38: 383–412.
(14) ———
1924. THE RELATION OF MICROÖRGANISMS TO THE DEVELOPMENT AND LONGEVITY OF FLIES. Amer. Jour. Trop. Med. 4: 85–107, illus.
(15) GUYÉNOT, E.
1917. RECHERCHES EXPÉRIMENTALES SUR LA VIE ASEPTIQUE ET LE DÉVELOPPEMENT D'UN ORGANISME (DROSOPHILA AMPELOPHILA) EN FONCTION DU MILIEU. DEUXIÈME PARTIE. Bul. Biol. France et Belg. 51: 137–330, illus.
(16) HARROW, B.
[1921]. VITAMINES; ESSENTIAL FOOD FACTORS. 219 p., illus. New York.
(17) HAWK, P. B., SMITH, C. A., and BERGEIM, O.
1921. THE VITAMINE CONTENT OF HONEY AND HONEY COMB. Amer. Jour. Physiol. 55: 339–348, illus.
(18) HOPKINS, F. G.
1920. THE EFFECTS OF HEAT AND AERATION UPON THE FAT-SOLUBLE VITAMINE. Biochem. Jour. 14: 725–733, illus.
(19) JOHNS, C. O., and FINKS, A. J.
1920. STUDIES IN NUTRITION. II. THE RÔLE OF CYSTINE IN NUTRITION AS EXEMPLIFIED BY NUTRITION EXPERIMENTS WITH THE PROTEINS OF THE NAVY BEAN, PHASEOLUS VULGARIS. Jour. Biol. Chem. 41: 379–389, illus.
(20) LOEB, J., and NORTHROP, J. H.
1916. NUTRITION AND EVOLUTION. SECOND NOTE. Jour. Biol. Chem. 27: 309–312.
(21) ——— and NORTHROP, J. H.
1917. ON THE INFLUENCE OF FOOD AND TEMPERATURE UPON THE DURATION OF LIFE. Jour. Biol. Chem. 32: 103–121, illus.
(22) MCCOLLUM, E. V., and DAVIS, M.
1915. THE INFLUENCE OF CERTAIN VEGETABLE FATS ON GROWTH. Jour. Biol. Chem. 21: 179–182, illus.
(23) ——— SIMMONDS, N., and PITZ, W.
1917. THE DIETARY DEFICIENCIES OF THE WHITE BEAN, PHASEOLUS VULGARIS. Jour. Biol. Chem. 29: 521–536, illus.
(24) ——— SIMMONDS, N., and PARSONS, H. T.
1919. THE DIETARY PROPERTIES OF THE PEA (VICIA SATIVA). Jour. Biol. Chem. 37: 287–301, illus.
(25) NORTHROP, J. H.
1917. THE RÔLE OF YEAST IN THE NUTRITION OF AN INSECT (DROSOPHILA). Jour. Biol. Chem. 30: 181–187.
(26) ———
1917. THE EFFECT OF PROLONGATION OF THE PERIOD OF GROWTH ON THE TOTAL DURATION OF LIFE. Jour. Biol. Chem. 32: 123–126.
(27) OSBORNE, T. B., and WAKEMAN, A. J.
1915. DOES BUTTER-FAT CONTAIN NITROGEN AND PHOSPHORUS? Jour. Biol. Chem. 21: 91–94.
(28) ——— and MENDEL, L. B.
1916. THE STABILITY OF THE GROWTH-PROMOTING SUBSTANCE IN BUTTER FAT. Jour. Biol. Chem. 24: 37–39.

(29) OSBORNE, T. B., and MENDEL, L. B.
1919. THE NUTRITIVE VALUE OF THE WHEAT KERNEL AND ITS MILLING PRODUCTS. Jour. Biol. Chem. 37: 557–601, illus.
(30) ——— WAKEMAN, A. J., and FERRY, E. L.
1919. PREPARATION OF PROTEIN FREE FROM WATER-SOLUBLE VITAMINE. Jour. Biol. Chem. 39: 35–46, illus.
(31) PORTIER, P.
1919. DÉVELOPPEMENT COMPLET DES LARVES DE TENEBRIO MOLITOR, OBTENU AU MOYEN D'UNE NOURRITURE STÉRILISÉE À HAUTE TEMPÉRATURE (130°). Compt. Rend. Soc. Biol. [Paris] 82: 59–60.
(32) SHERMAN, H. C.
1918. CHEMISTRY OF FOOD AND NUTRITION. Ed. 2, 454 p., illus. New York.
(33) VOEGTLIN, C., and MYERS, C. N.
1919. DISTRIBUTION OF THE ANTINEURITIC VITAMINE IN THE WHEAT AND CORN KERNEL. A CONTRIBUTION TO THE BIOLOGY OF THE ANTINEURITIC VITAMINE. Amer. Jour. Physiol. 48: 504–511, illus.
(34) WHITING, P. W.
1919. GENETIC STUDIES ON THE MEDITERRANEAN FLOUR-MOTH, EPHESTIA KÜHNIELLA ZELLER. Jour. Expt. Zool. 28: 413–445, illus.
(35) WOLLMAN, E.
1919. ÉLEVAGE ASEPTIQUE DE LARVES DE LA MOUCHE À VIANDE (CALLIPHORA VOMITORIA), SUR MILIEU STÉRILISÉ À HAUTE TEMPÉRATURE. Compt. Rend. Soc. Biol. [Paris] 82: 593–594.
(36) ———
1919. LARVES DE MOUCHE (CALLIPHORA VOMITORIA) ET VITAMINES. Compt. Rend. Soc. Biol. [Paris] 82: 1208–1210.
(37) ———
1922. BIOLOGIE DE LA MOUCHE DOMESTIQUE ET DES LARVES DE MOUCHE À VIANDE, EN ÉLEVAGES ASEPTIQUES. Ann. Inst. Pasteur 36: 784–788.

INHERITANCE OF RESISTANCE TO LEAF RUST, PUCCINIA TRITICINA ERIKSS., IN CROSSES OF COMMON WHEAT, TRITICUM VULGARE VILL.[1]

By E. B. MAINS, *Associate Botanist, Purdue University Agricultural Experiment Station, and Agent, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture;* C. E. LEIGHTY, *Agronomist in Charge of Eastern Wheat Investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture;* and C. O. JOHNSTON, *Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, stationed at Manhattan, Kans.*[2]

INTRODUCTION

Of the various cereal rusts, the stem rust caused by *Puccinia graminis* Pers. has received the most attention, on account of the very heavy losses caused by it in many regions.

The inheritance of resistance to this rust has been studied by Hayes, Parker, and Kurtzweil (*10*),[3] Aamodt (*1*, *2*), Melchers and Parker (*14*), Puttick (*18*), Waldron (*21*), Hayes and Aamodt (*11*), Harrington and Aamodt (*9*), Clark (*6*), Garber (*8*), and Dietz (*7*), in the United States. The inheritance of resistance to *Puccinia glumarum* (Schm.) Erikss. and Henn. has been studied most extensively in Europe by Biffen (*4*), Nilsson-Ehle (*16*), Armstrong (*3*), and Vavilov (*20*). Parker (*17*) has studied the inheritance of resistance of oats to crown rust, *Puccinia coronata* Corda. Mains and Leighty (*12*) have reported some results on the inheritance of resistance to the leaf rust of rye, *Puccinia dispersa* Erikss. and Henn.

Although studies made by Carleton (*5*), Vavilov (*20*), Melchers and Parker (*15*), Mains and Jackson,[4] and others, have resulted in the discovery of a number of varieties of wheat resistant to the leaf rust of wheat, *Puccinia triticina* Erikss., apparently no study has been made of the inheritance of resistance to the rust. As the leaf rust of wheat is, in many seasons, a factor of considerable importance in the production of wheat, especially in the soft-winter wheat areas of the United States, it has been the subject of considerable study during the last seven years. This investigation has resulted in the discovery of a number of varieties of wheat resistant to leaf rust. It also has been found that the leaf rust of wheat comprises several physiologic forms [4] to which these varieties differ in their susceptibility.

It was evident early that no one variety was likely to possess the desired rust resistance as well as other qualities which would make it suitable for the various sections of the country. In consequence, a knowledge of the manner in which resistance to the various physio-

[1] Received for publication Aug. 19, 1925; issued May, 1926. Cooperative investigations between the Indiana and Kansas Agricultural Experiment Stations and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[2] The writers wish to express their appreciation for the assistance rendered in various stages of the work by L. E. Compton and W. J. Sando of the Office of Cereal Investigations; and for assistance rendered and land and equipment provided by S. H. Essary, of the Tennessee Agricultural Experiment Station, and by L. E. Melchers, of the Kansas Agricultural Experiment Station.

[3] Reference is made by number (italic) to "Literature cited," p. 971.

[4] MAINS, E. B., and JACKSON, H. S. PHYSIOLOGIC SPECIALIZATION IN THE LEAF RUST OF WHEAT, PUCCINIA TRITICINA. Phytopathology 16:89–120. 1926.

logic forms of leaf rust is inherited became of great importance to the development of resistant varieties. The results obtained from a study of different hybrid combinations in the field at Washington, D. C., Knoxville, Tenn., La Fayette, Ind., and in the experiment station greenhouses at La Fayette, Ind., and Manhattan, Kans., are given in this paper.

MATERIAL AND METHODS

PARENT VARIETIES AND HYBRIDS

The results reported in this paper were obtained in experiments begun in 1920, when a series of hybrids was produced at Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., between several strains of Kanred and several other varieties of common wheat. The experiments then begun were designed in part to study the inheritance of resistance to leaf rust, but mainly to develop a wheat adapted to the humid winter-wheat area and resistant to this rust.

All of the important varieties of wheat now grown in this area are susceptible to one or more of the widely distributed forms of leaf rust. As a result, leaf rust usually is very prevalent on all of the wheat grown where humid conditions prevail. The amount of damage done by the rust varies considerably from season to season and from place to place. The crop seldom is ruined entirely but sometimes severe losses occur. Avoidance of such losses through the adoption of resistant varieties is an attractive proposition.

Several strains and varieties of the hard red winter wheats have been more or less resistant to leaf rust when grown in certain of the humid eastern sections of the country. However, wheats of this class are unadapted to these conditions and, as a rule, yield very poorly. Over most of the humid winter-wheat area, wheats of the soft red winter class are grown, although in some sections white-kerneled common wheat is given preference. The problem then is to combine the resistance of the one class with the factors in the other classes which make them adaptable.

The strains P762–9, P1066–1, and P1068–1, which were used as the resistant parents of the crosses made in 1920, originated from selections made at the Kansas Agricultural Experiment Station. (P762–9 is a selection of Kanred, and the others are practically identical with it in all respects, and so they will be referred to collectively as Kanred.) Kanred is widely grown and well adapted in Kansas and other States of the hard red winter-wheat area. Unfortunately, it has been found that Kanred sometimes is considerably susceptible to leaf rust, especially to most physiologic forms in the seedling stage under greenhouse conditions.

These unfavorable characteristics of Kanred having been determined, a different resistant parent was used in the crosses made in 1921. This was Malakoff, C. I. No. 4898, a variety quite similar in appearance to Kanred and belonging also to the hard red winter class. This strain has been found more satisfactory than Kanred in many respects, as will appear later.

The wheats crossed with Kanred and Malakoff were mainly of the soft red winter class, although certain other classes also were

used (Table 1). The varieties used for the most part were the ones that have been found best adapted to the humid areas.

In the spring of 1921, in the greenhouses at La Fayette, four crosses were made between varieties which were known to be reciprocally resistant and susceptible to several of the physiologic forms of leaf rust. These were made with the object of determining whether the resistance of two varieties to different physiologic forms could be combined, and to determine the possibilities of obtaining a variety resistant to all physiologic forms. In this paper these hybrids are called "hybrids between differentials."

The cross between Fulcaster and Kanred was made in the greenhouse at Manhattan, Kans., in the spring of 1922, the strain of Fulcaster used for the resistant parent being a selection discovered at that place and differing markedly in this respect from the variety in general.

The names of the varieties used, the agronomic or commercial class to which they belong, and the Office of Cereal Investigations (C. I.), and selection numbers appear in Table I.

TABLE I.—*Wheats used as parents of hybrids in studies on leaf rust, with the commercial class to which they belong and their Cereal Investigations (C. I.) and selection numbers*

Class and variety	Cereal Investigations No. [a]	Selection No.	Class and variety	Cereal Investigations No. [a]	Selection No.
Hard Red Winter (T. vulgare):			*Soft Red Winter (T. Vulgare)*—Continued.		
Banat		28	Penquite	5629	438
Crimean		26	Purplestraw	1915	340 and 269
Hussar	4843		Red Rock	5597	402
Kanred	5146	17	Rising Sun	5977	188
Malakoff	4898	19	Rudy	5625	434
Minturki	6155	333	Selection	(In 5639)	449
P762–9	5146	264	Sol	4139	1111
P1066–1	5879	267	Super	5544	365
P1068–1	5880	259	Treasure	5861	648
Weissenberg	1563		Trumbull	5657	469
Soft Red Winter (T. vulgare):			Valley	5658	471
Barletta	3297	351	Virginia	5606	412
Blue Ridge	4887	24	*Common White (T. vulgare):*		
China	5669	483	Fortyfold	4230	429
Early Noe	5856	646	Genesee Giant	1744	38
Early Ripe	5641	452	Gold Coin	5645	456
Fulcaster		252	Junior No. 6	6971	1226
Fulcaster		Kan. 55	Mammoth Amber	3551	1061
Fultzo-Mediterranean	5677	491	Martin	4636	1225
Gladden	5644	455	New Amber Longberry	1973	41
Grandprize	5627	436	*Club (T. compactum):*		
Gypsy	5646	457	Hybrid 128	4512	448
Harvest King	5647	458	Little Club	4081	444
Jersey Fults	5581	385	*Hard Red Spring (T. vulgare):*		
Jolly Farmer	5858	647	Prelude		352
Leap		244	Minn. 924		309
Leap	5684	498	Selection in Royalton	(In 4968)	314
Mealy	5650	462	Norka	4377	
Mediterranean	5588	392	*Unclassified:*[b]		
Mediterranean	5651	463	(Unnamed)	3756	
Michigan Amber	6980	29	Do	3778	
Minessa	6154	417	Webster	3780	
Nigger	5652	464			
Pennsylvania No. 44	6693	22			

[a] Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[b] These wheats are described by MAINS, E. B., and JACKSON, H. S. PHYSIOLOGIC SPECIALIZATION IN THE LEAF RUST OF WHEAT, PUCCINIA TRITICINA. Phytopathology 16: 89–120. 1926.

RUST IN FIELD NURSERIES

In the field nurseries at Washington, Knoxville, and La Fayette the infection of the hybrids and parents resulted from the rust naturally occurring in those localities. In most instances, leaf rust was so abundant that susceptible varieties were heavily infected and a very good test of reaction to the rust was obtained. Under such conditions it was not possible to control the physiologic forms of the rust by which the hybrids were infected.

In taking notes upon hybrids in the field nurseries, infection was determined according to the scale in use in the Office of Cereal Investigations, by which it is estimated in percentages of the number of uredinia actually formed compared with the number possible. In addition to the reduction in number of uredinia formed on resistant plants, there was also a reduction in size and an increase in the amount of chlorosis and necrosis, with and without uredinia. Thus infections noted as representing from 0 to 15 per cent were accompanied by a considerable number of chlorotic and necrotic spots without uredinia, the uredinia produced being mostly small and in similar spots. This necrosis and chlorosis decreased, practically disappearing when 50 to 65 per cent uredinia were produced.

METHODS IN GREENHOUSE STUDIES

The methods of inoculation employed in the greenhouse studies have been described elsewhere[5] (*12*, *13*, *15*). For the most part, only young plants having two to four leaves were inoculated. In the studies of the Fulcaster-Kanred cross, inoculations were made at three stages of development. These were: (1) The seedling stage, in which inoculations were usually made a month or more after sowing, when four or five leaves were out; (2) the shooting or jointing stage, in which the plants had passed the period of slow growth corresponding to the winter period out-of-doors, and were growing rapidly and preparing to head; and (3) the heading stage, in which the plants were inoculated two or three days after the heads had emerged from the boot.

Notes were taken when infection had reached its maximum development, about 14 to 21 days after inoculation, depending upon light and temperature conditions. The plants were classified according to the type of infection which developed. Five different classes of reaction to rust have been recognized, based upon the types of infection produced (*12*, *13*, *19*). These were as follows:

Class of reaction.	Type of infection.
0. Highly resistant	No uredinia formed; small flecks, chlorotic or necrotic areas more or less prevalent.
1. Very resistant	Uredinia few, small, always in small necrotic spots. More or less necrotic areas produced without the development of uredinia.
2. Moderately resistant	Uredinia fairly abundant, of moderate size, always in necrotic or very chlorotic spots. Necrotic spots seldom without uredinia.
3. Moderately susceptible	Uredinia fairly abundant, of moderate size. No necrosis produced, but sometimes slight chlorosis immediately surrounding the uredinia.

[5] MAINS, E. B., and JACKSON, H. S. PHYSIOLOGIC SPECIALIZATION IN THE LEAF RUST OF WHEAT, PUCCINIA TRITICINA. Phytopathology 16: 89–120. 1926.

Class of reaction.	Type of infection.
4. Very susceptible	Uredinia abundant, large. No necrosis or chlorosis immediately surrounding the uredinia. Infected areas sometimes occurring as green islands surrounded by a chlorotic ring.

The physiologic form of leaf rust [6] employed in the greenhouse studies depended upon the hybrid which was being studied. With but one exception, each of the various cultures employed was started from a single urediniospore and, in consequence, was a pure culture of the physiologic form. The culture used in the study of the Fulcaster-Kanred cross consisted of a mixture of two physiologic forms, one of which was present in such small numbers as not to interfere with the results.

EXPERIMENTAL RESULTS

Experiments were conducted in nurseries under field conditions in Virginia, Tennessee, and Indiana; and under greenhouse conditions at La Fayette, Ind., and Manhattan, Kans.

FIELD RESULTS

Two groups of hybrids were grown in the field nurseries These comprise the progenies resulting from crosses between the resistant parents, Kanred and Malakoff, respectively, and various other varieties of common wheat.

Results With Kanred Hybrids

Data on the reaction of the F_1 and F_2 progeny of hybrids between strains of Kanred and various other varieties of common wheat (*Triticum vulgare*) are given in Table II. The F_1 was grown only in the field nursery at Arlington Experiment Farm (W). In most cases the F_1 plants were nearly or quite as badly rusted as the susceptible parent. For instance, in the cross P1068–1 × Purplestraw, the F_1 plants were rusted from 80 to 95 per cent. Purplestraw, the susceptible parent, also was rusted from 80 to 95 per cent, whereas P1068–1 had only from 5 to 15 per cent of rust. In 15 of the 31 cases for which data are available the rust percentage for the F_1 is exactly the same as that for the susceptible parent (in one case the F_1 is slightly higher), indicating full dominance of susceptibility. In seven additional cases the F_1 is only slightly less rusted than the susceptible parent, the difference being so small (5 to 10 per cent) as hardly to indicate the intermediate condition. In the eight remaining cases the differences are somewhat greater. In some of these it appears that true intermediacy exists, as in the cross P762–9 × Harvest King, the F_1 of which is rusted from 40 to 50 per cent, the resistant parent (P762–9) from 0 to 25 per cent, and the susceptible parent from 75 to 85 per cent. The crosses P1066–1 × Trumbull, P762–9 × Gold Coin, and P762–9 × China, were also distinctly intermediate in the F_1. It appears, therefore, from the data at hand that in the F_1 of hybrids between Kanred and other varieties of common wheat, susceptibility to leaf rust was sometimes dominant in

[6] Mains, E. B., and Jackson, H. S. Physiologic specialization in the leaf rust of wheat, Puccinia triticina. Phytopathology 16: 89–120. 1926.

inheritance and sometimes more or less definitely intermediate, depending on the susceptible parent used.

TABLE II.—*Reaction to leaf rust of wheat (Puccinia triticina) of the F_1, the F_2, and the parents of hybrids between strains of Kanred and the different wheat varieties in field nurseries at Arlington Experiment Farm near Washington, D. C., (W), Knoxville, Tenn. (K), and La Fayette, Ind. (L), in 1921 and 1922*

Parent or hybrid combination	Location	Percentage of infection			Numbers of F_2 segregates in 1922 showing different percentages of infection							
		F_1	Parents		0–14	15–24	25–34	35–49	50–64	65–74	75–100	Total
		1921	1921	1922								
P1068–1	W		5–15	0– 35								
	K			5– 15								
	L			15– 35								
P1068–1×Purplestraw	W	80–95			0	1	4	3	40	70	21	139
	K				2	4	8	7	54	54	28	157
	L				6	3	50	41	52	5	1	158
Purplestraw	W		80–95	70–100								
	K			50– 75								
	L			50– 65								
P1068–1×Leap (244)	W	40–60			1	1	9	4	68	52	20	155
	W	65–75										
	K				3	4	4	2	12	6	3	34
	L				1	0	5	2	15	22	19	64
	L				5	3	39	45	27	1	0	120
Leap (244)	W		65–75	80–100								
	W		65–75									
	K			50– 75								
	L			65– 90								
	L			50								
P1068–1×Fulcaster (252)	W	40–75			0	10	7	1	90	44	19	171
	K				38	11	80	2	42	0	0	173
	L				3	7	35	29	0	0	0	83
Fulcaster (252)	W		40–75	70–100								
	K			25– 50								
	L			25– 50								
P1066–1	W		0–15	5– 50								
	K			5– 15								
	L			15– 35								
P1066–1×Barletta	W	65–85			0	0	1	3	24	43	40	111
	K				0	6	13	1	36	1	28	85
	L				0	1	13	22	61	11	2	110
Barletta	W		75–85	70– 90								
	K			75– 90								
	L			65– 90								
P1066–1×Prelude	W	50–65			0	2	34	2	90	8	2	138
	K				0	1	19	0	26	0	22	68
	L				0	0	15	23	72	8	0	118
Prelude	W		50–75	50– 75								
	K			80– 90								
	L			65– 75								
P1066–1×Super	W	30–40			0	1	5	0	70	43	9	128
	K				0	13	34	0	82	0	25	154
	L				0	0	15	29	9	57	0	110
Super	W		30– 50	75–100								
	K			60–80								
	L			60								
Purplestraw×P1066–1	W	75–85			0	2	4	0	46	46	33	131
	K				3	3	24	0	92	12	24	158
	L				1	3	7	0	46	39	0	96
P1066–1×Jersey Fultz	W	50–65			0	1	3	0	69	72	31	176
	K				0	0	6	0	107	10	40	163
	L				2	4	4	7	46	39	19	121
Jersey Fultz	W		50– 70	65–100								
	K			80–100								
	L			65– 75								
P762–9	W			5– 50								
	K			5– 15								
	L			15– 35								
P762–9×Red Rock	W	70–90			0	1	7	0	62	64	14	148
	K				5	2	9	2	89	22	30	159
	L				5	9	9	9	42	40	20	134
Red Rock	W		85– 95	70– 90								
	K			75								
	L			75								

TABLE II.—*Reaction to leaf rust of wheat (Puccinia triticina) of the F_1, the F_2, and the parents of hybrids between strains of Kanred and the different wheat varieties in field nurseries at Arlington Experiment Farm near Washington, D. C., (W), Knoxville, Tenn. (K), and La Fayette, Ind. (L), in 1921 and 1922*—Continued

Parent or hybrid combination	Location	Percentage of infection			Numbers of F_2 segregates in 1922 showing different percentages of infection							
		F_1	Parents		0–14	15–24	25–34	35–49	50–64	65–74	75–100	Total
		1921	1921	1922								
P762-9×Mediterranean, C.I. 5588	W	50–60			0	0	2	0	59	57	13	131
	K				0	4	15	0	118	17	9	163
	L				0	7	22	20	68	38	4	159
Mediterranean, C. I. 5588	W		60– 75	65– 90								
	K			75– 90								
	L			65– 75								
P762-9×Penquite	W	50–60			0	0	2	0	24	49	28	103
	L				1	6	9	10	62	48	17	153
Penquite	W		40– 50	65–100								
	L			70– 90								
P762-9×Sel. 449 (in C. I. 5639)	W	65–75			0	0	1	0	25	65	52	143
	K				0	0	5	0	100	2	53	160
	L				2	0	4	2	32	44	48	132
Sel. 449	W		75– 85	70–100								
	K			70– 90								
	L			75–100								
P762-9×Harvest King	W	40–50			0	0	0	0	18	47	23	88
	K				0	0	4	1	89	8	52	154
	L				1	1	6	3	31	46	46	134
Harvest King	W		75– 85	65– 75								
	K			70– 90								
	L			75– 90								
P762-9×Mealy	W	40–50			0	1	2	0	27	49	20	99
	L				10	2	8	8	69	51	5	153
Mealy	W		50– 70	70–100								
	L			70–100								
P762-9×Nigger	W	75–85			0	0	0	0	14	23	15	52
	L				0	0	2	2	15	33	7	59
Nigger	W		75– 90	70–100								
	L			65– 75								
P1066-1×Trumbull	W	30–50			1	2	9	0	31	36	23	102
Trumbull	W		50– 65	65– 90								
P1066-1×Valley	W	40–50			0	2	13	1	54	30	5	105
	K				0	0	1	0	8	1	2	12
	L				8	1	7	7	40	34	11	108
Valley	W		40– 50	65– 75								
	K			70– 90								
	L			65– 75								
P1066-1×Fultzo - Mediterranean.	W				0	0	2	3	106	50	16	177
	K				0	2	1	0	13	0	12	28
	L				0	0	8	8	40	31	11	98
Fultzo-Mediterranean	W			65– 90								
	K			80–100								
	L			50– 65								
Purplestraw×P1066-1	W				0	2	7	1	103	40	9	162
P1066-1×Rudy	W				0	0	1	0	58	23	2	84
Rudy	W			65– 90								
P1066-1×Grandprize	W				1	4	16	7	75	13	1	117
	K				1	5	7	0	34	7	3	57
	L				1	7	19	11	50	13	0	101
Grandprize	W			65– 75								
	K			65– 75								
	L			50– 65								
P1066-1×Early Ripe	W				0	1	5	2	58	18	5	89
	L				1	0	5	12	17	12	0	47
Early Ripe	W			65– 75								
	K			50– 65								
P1066-1×Early Noe	W				0	0	7	1	34	11	3	56
Early Noe	W			70–100								
P1066-1×Jolly Farmer	W				0	3	9	6	65	19	0	102
Jolly Farmer	W			65– 75								
P1066-1×Gladden	W				0	6	17	1	109	9	0	142
	K				0	0	5	1	63	10	19	98
	L				0	0	5	10	54	42	3	114
Gladden	W			50– 75								
	K			75–100								
	L			65								
P762-9×Gold Coin	W	30–40			0	0	9	3	47	12	2	73
Gold Coin	W		75– 85	65– 90								
P762-9×Mediterranean, C. I. 5651	W	65–75			0	0	6	1	47	11	7	72

TABLE II.—*Reaction to leaf rust of wheat (Puccinia triticina) of the F_1, the F_2, and the parents of hybrids between strains of Kanred and the different wheat varieties in field nurseries at Arlington Experiment Farm near Washington, D. C., (W), Knoxville, Tenn. (K), and La Fayette, Ind. (L), in 1921 and 1922*—Continued

Parent or hybrid combination	Location	Percentage of infection			Numbers of F_2 segregates in 1922 showing different percentages of infection							
		F_1	Parents		0–14	15–24	25–34	35–49	50–64	65–74	75–100	Total
		1921	1921	1922								
Mediterranean, C. I. 5651	W		65– 75	65– 75								
P762-9×China	W	30–40			3	30	59	31	47	0	0	170
	K				0	0	5	0	120	9	28	162
	L				0	0	11	11	55	60	2	139
China	W		50– 65	30– 50								
	K			75								
	L			65								
P762-9×Minturki	W				0	1	36	0	105	19	1	162
Minturki	W			50								
P762-9×Minessa	W				14	19	110	13	11	0	0	167
Minessa	W			50								
P762-9×C. I. 6142	W				0	1	8	0	104	38	7	158
C. I. 6142	W			50– 75								
P762-9×Gypsy	W	50–65										
	K				0	3	4	0	94	40	21	162
	L				1	4	13	13	76	42	12	161
Gypsy	W		50– 65									
	K			65– 75								
	L			75								
P762-9×Minn. 924	W	30–50										
	K				7	0	7	0	99	49	10	172
	L				6	1	28	4	52	46	24	161
Minn. 924	W		30– 50									
	K			50– 65								
	L			75								
P1068-1×Mealy	W	65–75										
	K				0	0	0	0	11	8	10	29
	L				0	0	1	2	9	30	26	68
Mealy	W		65– 75									
	K			100								
	L			50– 65								
P1068-1×Virginia	W	65–75										
	K				0	0	1	0	49	25	47	122
	L				0	0	2	6	58	56	27	149
Virginia	W	65–75										
	K			80– 90								
	L			75– 90								
P1068-1×Genesee Giant	W	50–65										
	K				0	0	0	0	45	35	71	151
	L				0	0	10	11	80	32	7	140
Genessee Giant	W	50–65										
	K			80–100								
	L			50– 75								
P762-9×Selection in Royalton	W	40–65										
	L				3	0	9	5	29	35	26	108
Selection in Royalton	W	40–65										
	L			75								
P1066-1×Forty fold	W	75–85										
	L				1	0	3	0	45	47	41	137
Fortyfold	W		75– 85									
	L			75								
P1068-1×Little Club	W	65–75										
	L				0	0	0	3	35	41	40	119
Little Club	W		65– 75									
	L			100								
P1068-1×Treasure	L				0	0	9	6	56	32	16	119
Treasure	L			75								
P1068-1×New Amber Longberry	W	40–50										
	L				0	0	1	2	48	27	0	78
New Amber Longberry	W		40– 50									
	L			65– 75								
P1066-1×Hybrid 128	W	75–85										
	L				0	0	2	5	29	57	55	148
Hybrid 128	W		85–100									
	L			100								
P762-9×Rising Sun	L				2	0	10	9	12	18	10	61
Rising Sun	L			75–100								

The percentage of rust on the F_1 varied considerably in the different crosses. The lowest was from 30 to 40 per cent on the P1066–1 × Super and the P762–9 × Gold Coin and China crosses. The highest was from 80 to 95 per cent on the P1068–1 × Purplestraw. The susceptible parents, Super, Gold Coin, and Purplestraw, were rusted from 30 to 50, from 75 to 85, and from 80 to 95 per cent, respectively. Although the relationship is not perfect, taking the data as a whole, there is a high degree of correlation between the percentage of rust of the susceptible parent and that of the F_1.

In 1922 the F_2 generation of the crosses between Kanred and other varieties of wheat was grown in field nurseries at Arlington Experiment Farm (W), Knoxville (K), and La Fayette (L). The data recorded at these places on the F_2 segregates and the parents of the different hybrids also are given in Table II.

In order to facilitate their presentation, the data on the percentage of rust on the individual F_2 plants have been grouped in seven classes, opposite the letter, given above, designating the location of the nursery. The grouping adopted is justified by these reasons. The first group, containing percentages from 0 to 14, is well within the upper limits of reaction of the resistant parent. It contains few individuals, and all of these actually were in the 5 and 10 per cent classes. The second group, containing percentages from 15 to 24, contains but two individuals in class 20, all others being in the 15 per cent class. The third group, representing from 25 to 34 per cent of infection, contains only seven individuals in the 30 per cent class, all others being in the class of 25 per cent. Group four, covering from 35 to 49 per cent, contains 27 individuals in the 40 per cent class, and all the others in the 35 per cent class. The fifth group, covering from 50 to 64 per cent of infection, has all individuals in the 50 per cent class. Group six, covering from 65 to 74 per cent, has all individuals, except one, in the 65 per cent class. Group seven, showing from 75 to 100 per cent of infection, contains the highly susceptible individuals. Most of these were in the 75 per cent class, although some of the progenies contained individuals in other classes, 19 at 80 per cent, 95 at 90, and 70 at 100 per cent.

When the distributions of the F_2 progenies are examined, it is evident that segregation for resistance to rust has occurred. In general, highly resistant, highly susceptible, and intermediate individuals are found in the different populations grown at all three of the stations. In several cases the resistant, intermediate, and susceptible individuals approximate some simple Mendelian ratio, when parental susceptibility is used as a limit to the classes. But no ratio has been found to fit all cases. In fact, two populations of the same cross grown at different stations sometimes seemed to have segregated in different ratios, this due probably to differences in the physiologic forms of rust occurring in these localities. After considerable study of the data it does not seem possible to assign the segregates of the different populations to any definite ratio. Neither does it seem profitable to attempt to work out separate ratios for the different distributions obtained. It is evident that segregation has occurred, but no conclusion has been arrived at from consideration of the field-grown material as to the number of factors involved in the inheritance of rust resistance in these hybrids.

It is evident that when a somewhat resistant parent is combined with Kanred, which also possesses resistance of some kind, the segregates, on the whole, are less rusted. More individuals are found in the less-rusted groups, and few or none in the heavily rusted groups. Segregates rarely are found with a higher percentage of rust than that of the more susceptible parent, although a few cases of this kind occur.

The results obtained in the field are made complex by several factors. One of these is the existence of several physiologic forms of rust at the stations where the plants were grown. Although the behavior of these forms on Kanred is not definitely known, there is some evidence that this variety is more heavily rusted by certain ones than by others. It is possible, therefore, that differences in the proportion of given physiologic forms in the rust mixture present may result in differences in percentage of rust on Kanred and segregates at different places. There also is evidence that environmental factors also may modify the reaction of Kanred to leaf rust. This apparently is most marked in the fairly high susceptibility of Kanred in the greenhouse to cultures of rusts from localities where it was resistant in the field.

Climatic conditions may favor or retard the development of leaf rust in any year or any place. If conditions are such that rust does not develop until late in the season, many of the plants will have reached such a stage of maturity that infection does not occur, or they may be so far along in their development that the rust spores do not affect the plants in a normal way. In this case plants actually susceptible may seem resistant. In several of the hybrids one of the parents is early maturing. This results in a segregation for earliness in the F_2 and a possibility that notes for susceptibility are misleading on this account.

There enters also the matter of adaptation of the parents. All of the hybrids having Kanred or Malakoff as one of the parents have this factor entering in. As stated, Kanred and Malakoff are rather poorly adapted to the area in which the hybrids were grown. The hybrids were made in the effort to combine the rust resistance of Kanred and Malakoff with the adaptation of the other parents. There must be some influence in the hybrids from whatever factors are responsible for this adaptability, as it is generally recognized that, other conditions being equal, rust development is proportional to the vigor of the host. The evidence at hand indicates that there is no linkage of external characters and resistance, for all types of resistant plants have been found.

Results with Malakoff Hybrids

The hybrids in which Malakoff was used as the resistant parent were made in 1921. The F_1 and F_2 were grown in the field nursery only at Arlington Experiment Farm, although a few of F_1 and all of F_2 were grown in the greenhouse at LaFayette, Ind. The data obtained in the field nursery at Arlington Experiment Farm are given in Table III.

In three of these crosses, Malakoff × Weissenberg, Malakoff × Kanred, and Malakoff × Crimean, the F_1 plants were classified as having infection from a trace (T) to 15 per cent, a trace, and a trace to 15

per cent, respectively. Probably this should be considered as true dominance of resistance, although certain of the parental plants were rusted more than the F_1 plants, thereby indicating a greater resistance in the F_1 than in the parents. The parental plants were rusted as follows: Malakoff 0 to 35 per cent, Weissenberg T to 25, Kanred 25 to 35, and Crimean 15 to 50 per cent. The upper limit for Malakoff probably should be considered exceptional, as most of the plants were rusted only 0 to 15 per cent. The F_1 of all other hybrids with Malakoff listed in this table were intermediate in reaction. Unlike the hybrids with Kanred, in which susceptibility usually was dominant or nearly so in the F_1, with only a few definite cases of intermediacy, resistance in these hybrids with Malakoff sometimes is dominant, with intermediacy as the usual condition. Data on the F_2 generation of the hybrids with Malakoff obtained in the field nursery at Arlington Experiment Farm also are given in Table III. In this table the numbers of F_2 segregates are given as actually recorded, except in the case of those having a trace, 5 per cent, or 10 per cent of rust, where they are grouped in the column, T–10 per cent.

TABLE III.—*Reaction to leaf rust of wheat (Puccinia triticina) of the F_1, the F_2, and the parents of hybrids between Malakoff and different wheat varieties in the field nursery at Arlington Experiment Farm, in 1922 and 1923*

Parent or hybrid combination	Percentage of infection			Numbers of F_2 segregates in 1923 showing different percentages of infection								
	F_1	Parents										
	1922	1922	1923	T–10	15	25	35	50	65	75	100	Total segregates
Malakoff		0–35	T–35									
Malakoff×Mammoth Amber	35–50			7	9	24	13	9	13	20	23	118
Mammoth Amber		50–75	100									
Malakoff×Weissenberg	T–15			12	18	18	1	4	13	0		66
Weissenberg		T–25	50–75									
Malakoff×Kanred	T.			93	12	14	6	7				132
Kanred		25–35	25–50									
Malakoff×Pennsylvania No. 44	25–50			63	19	21	14	20	16	15	8	176
Pennsylvania No. 44		50–65	50–75									
Malakoff×Junior No.6	35–65			0	3	14	15	28	51	40	2	153
Junior No. 6		65–75	65–75									
Malakoff×Michigan Amber	25–50			16	9	46	19	4	23	8	5	130
Michigan Amber		50–65	100									
Malakoff×Blue Ridge	35–50			50	14	28	25	4	15	17	3	156
Blue Ridge		50–75	75–100									
Malakoff×Martin	25–50			5	5	13	16	15	26	30	34	144
Martin		50–65	100									
Malakoff×Sol	15–25			0	7	20	8	12	20	25	58	150
Sol		35–50	100									
Malakoff×Banat	35–50			22	12	30	22	13	27	19	10	155
Banat		65	75									
Malakoff×Crimean	T–15			9	49	50	9	17	21	19	14	188
Crimean		15–50	75–100									

As in the crosses between Kanred and other wheats, segregation for rust resistance is evident in the F_2 of these crosses having Malakoff as one of the parents. In every family are found highly resistant, intermediate, and, when one of the parents is highly susceptible, highly susceptible individuals. By arbitrarily fixing limits for the groups, and combining or separating the numbers under the different rust percentages, Mendelian ratios can be determined for the different families. In most families the segregates can be assigned in this way

to the simple monohybrid ratios, 3 : 1, 1 : 3, and 1 : 2 : 1. Segregation in the cross, Malakoff × Pennsylvania No. 44, however, may be according to the dihybrid ration 9 : 3 : 3 : 1, thus accounting for the eight individuals recorded as more rusted than either parent. Although segregation in the several families apparently has taken place, and although there may be some basis in fact for the arbitrary groupings required, detailed Mendelian analysis of the data in Table III must, under the circumstances, appear artificial and will not be undertaken. This procedure is warranted, inasmuch as data are presented elsewhere in this paper which show that when complicating factors, such as occur in the field, are eliminated, inheritance of resistance is a definite fact and is Mendelian in character.

It is apparent in these data, as in those from the earlier series of hybrids, that when both parents are comparatively low in rust infection, all of the segregates usually are comparatively low; also, that when one parent approaches or equals 100 per cent, the segregates likewise will approach or equal 100 per cent. F_2 individuals seldom are rusted more than the susceptible parent. The few cases where this does occur may be ascribed to fluctuation or the effect of the complex of environmental factors, rather than to the condition of transgressive inheritance.

In the crosses involving Weissenberg and Kanred, both of which show some resistance, there seems to be a cumulative effect of the resistance possessed by them and by Malakoff, the other parent in each case, which results in the F_2 segregates being only slightly rusted. In the cross with Weissenberg, 48 of the 66 individuals were rusted 25 per cent or less. In the cross with Kanred, 93 of the 132 individuals were rusted only from a trace to 10 per cent. In the cross with Pennsylvania No. 44 the results are somewhat similar, although there are more individuals with high rust percentages and even a few more rusted than Pennsylvania No. 44. When the late-maturing highly susceptible varieties Martin and Sol are combined with Malakoff, which is also rather late-maturing, a piling up of the segregates in the high-rust classes is very evident.

Results With the F_3 Generation of Kanred and Malakoff Hybrids

Several hundred F_3 families were grown from plants selected in the F_2 segregates of the crosses listed above. For the most part plants combining low rust infection and other desired characters were selected, but in some of the crosses all of the F_2 plants were continued. The F_3 plants of the hybrids with Kanred were grown in field nurseries at Arlington Experiment Farm and at Knoxville. Data on these are given in Tables IV and V. The F_3 plants of the hybrids with Malakoff were grown in the field nursery at Arlington Farm. Data on these are given in Table VI. In these nurseries the plants were spaced the same as in the nurseries where the F_1 and F_2 generations were grown. Usually from 20 to 50 plants of each family were grown, although in some cases the number was necessarily smaller on account of lack of seed. The rust notes were taken on the family as a whole, not on individual plants. The notes, then, represent the range of rust reaction in the family, but do not furnish any information as to the distribution of the plants within the family in reference to susceptibility.

TABLE IV.—*Distribution of F_3 families of crosses between Kanred and other varieties of common wheat, classified according to reaction to leaf rust in the field nursery at Arlington Experiment Farm in 1923, in relation to the infection of the F_2 parental plants*

Percentage of F_2 parent infection	Numbers of F_3 families						
	Homozygous in rust reaction, by infection subclasses			Heterozygous in rust reaction, by infection subclasses			Total
	Low	Medium	High	Low to medium	Medium to high	Low to high	
5		1					1
10	1						1
15	20	10		7		4	41
25	25	19		22	2	10	78
35	1	3			1	3	8
50	7	44	6	16	19	17	109
65	2	27	7	12	23	11	82
75		12	5	1	12	3	33
90		1				1	2
100		1	1		5	3	10
Total	56	118	19	58	62	52	365

TABLE V.—*Distribution of F_3 families of crosses between Kanred and other varieties of common wheat, classified according to reaction to leaf rust in the field nursery at Knoxville, Tenn., in 1923, in relation to the infection of the F_2 parental plants*

Percentage of F_2 parent infection	Numbers of F_3 families						
	Homozygous in rust reaction, by infection subclasses			Heterozygous in rust reaction, by infection subclasses			Total
	Low	Medium	High	Low to medium	Medium to high	Low to high	
5	17	1		5		2	25
10	4			6		2	12
15	7	4	1	14	3	13	42
25	5	3	2	13	7	16	46
35	1				2	2	5
50				1		3	4
Total	34	8	3	39	12	38	134

TABLE VI.—*Distribution of F_3 families of crosses between Malakoff and other varieties of common wheat, classified according to reaction to leaf rust in the field nursery at Arlington Experiment Farm in 1924, in relation to the infection of the F_2 parental plants*

Percentage of F_2 parent infection	Numbers of F_3 families						
	Homozygous in rust reaction, by infection subclasses			Heterozygous in rust reaction, by infection subclasses			Total
	Low	Medium	High	Low to medium	Medium to high	Low to high	
0–T	7						7
5	12			1			13
10	8	1		5			14
15	21	6	1	3			31
25	14	5	1	11			31
35	5	3				1	9
50	7	6		3			16
65	6	5	2	1			14
75	5	10		2			17
100	7	11		1		1	20
Total	92	47	4	27		2	172

In order to facilitate the presentation of the data it has been assumed, arbitrarily, that a family in which the range of infection is 25 per cent or less is homozygous, and that a family in which the range is more than 25 per cent is heterozygous. Within each of these two major classes three arbitrary subclasses have been established.

In the homozygous class these are: "Low," including those families in which the range of rust infection percentage is between 0 and 35; "medium," including those families in which the range is between 50 and 65 per cent (including also those with ranges recorded as 25–50, 35–60, 50–75, and 65–75 per cent); and "high," including those families in which the range is from 75 to 100 per cent.

In the heterozygous class the subclasses are: "Low to medium," including those families in which the range is more than 25 per cent, and with the lower limit in the "low" subclass and the upper in the "medium" subclass [i. e. (0–35 per cent) to (50–65 per cent)]; "medium to high" [i. e. (50–65 per cent) to 75–100 per cent)], and "low to high" [i. e. (0–35 per cent) to (75–100 per cent)], which are similarly limited with respect to the subclasses described. The numbers of families in these 6 subclasses are then distributed in the tables in proper relation to the percentage of rust infection of the F_2 parent.

By means of this arbitrary system of classification, a distribution has been obtained which gives a fair idea of the data recorded. The classification probably is as nearly accurate as the original data. As already pointed out, the reaction to leaf rust shown by a plant growing in the field is dependent not only on the inherent qualities of the plant itself, but also on many external conditions. The percentage of infection recorded is the product of the interaction of internal and external forces. The plants selected in the F_2 for low rust infection may be inherently resistant, or, for some reason or other, may have escaped rust up to the time the observation was made. In the next generation the progeny are subjected to more or less different environmental conditions, and the physiologic form or mixture of forms of rust prevalent in the field may be different from those of the preceding year. As a result of these and perhaps other influences, the inheritance of leaf-rust resistance in plants grown in the field seems to be impossible of accurate determination.

Although a high degree of accuracy is not claimed for either the classification or the original data, it is believed that the distributions given in Tables IV, V, and VI have considerable significance from the standpoint of inheritance of resistance and, consequently, in connection with breeding for resistance to leaf rust. The tables show that many of the F_2 plants, low in rust infection, produced F_3 families also low. Other F_2 plants only slightly rusted produced families showing low infection, or families apparently segregating from low to medium infection; a few, however, produced families showing high infection, or segregating from medium to high, or low to high. A decided tendency also is apparent in the crosses with Kanred (Tables IV and V) for the F_2 plants showing medium infection (50 and 65 per cent) to produce F_3 families of medium and high infection, or segregating from medium to high, and low to high. The highly infected F_2 plants of these crosses have a tendency to produce families medium or high, and segregating from medium to high or low to high in rust infection.

The data in Table VI on the crosses with Malakoff show a decided tendency for all F_2 plants to produce families low in rust infection, irrespective of the reaction of the parent plant. This is doubtless due, in large part at least, to conditions existing in 1924, when rust appeared late and developed slowly. It was observed that leaf rust did not overwinter to the usual extent in the winter of 1923–24. The spring of 1924 was rainy, cool, and late. Conditions favorable for the development of leaf rust did not occur until late in the season, and many of the families were too far along for normal infection. At Knoxville practically no rust developed, so no data on resistance could be obtained there and no selection could be made for this quality. As a consequence the number of F_3 families of the crosses with Malakoff in the low, medium, and low to medium subclasses probably is abnormally high.

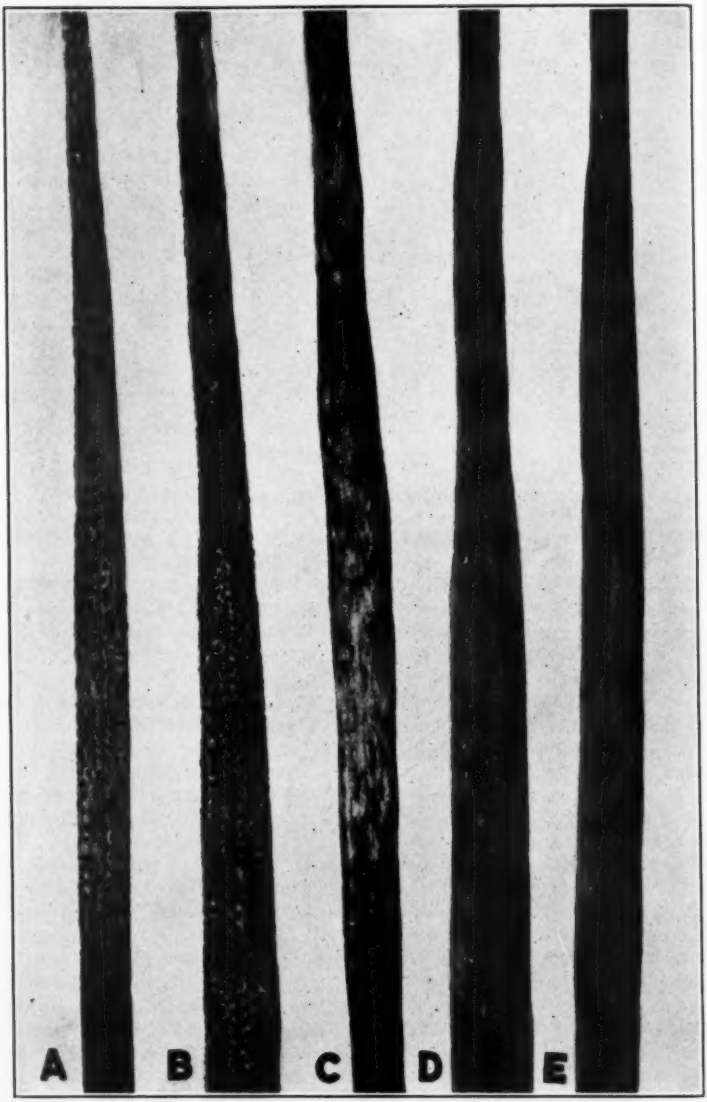
It is apparent from the data given that many of the F_2 plants selected for low rust infection have transmitted this characteristic to their progeny. This is of importance in connection with the attempt to breed for rust resistance.

GREENHOUSE RESULTS

Hybrids Between Resistant Fulcaster and Kanred

Among the crosses made at Manhattan in the spring of 1922 was one between Kanred and selections from Fulcaster which had proved highly resistant to the culture of leaf rust in use at that place. These selections of Fulcaster were interesting, in that the first seedling leaf often showed a rust reaction belonging in class 3, while the later seedling leaves were immune or very highly resistant, being in reaction class 0 to 1. When plants of these Fulcaster selections were in the shooting stage most of them were in class 0, showing only rather indistinct flecking (pl. 1, E). When innoculated in the heading stage the high resistance exhibited in the shooting stage was maintained. An occasional well-developed uredinium occurred on these plants, due to a slight contamination of the culture of physiologic form 9 by form 3. However, there was no difficulty in distinguishing these few uredinia from the slight flecking characteristic of the reaction of these selections to form 9. In contrast, a susceptible line of Fulcaster selected from the same source (Fulcaster Ks. 55) as that from which the resistant lines were obtained, showed high susceptibility (class 4) in all stages of development. Apparently, however, uredinia were not produced as abundantly in the shooting and heading stages as in the seedling. A few plants also exhibited a slight amount of flecking at these stages. Two families, Nos. 26 and 33, developed from plants originally thought to be resistant, proved to be moderately susceptible in all stages, but produced slightly fewer uredinia in the shooting and heading stages than in the seedling stage.

In this study Kanred was very similar in its action to the susceptible strains of Fulcaster, being highly susceptible in all stages (class 4), showing even more abundant production of uredinia than the susceptible strains of Fulcaster (pl. 1, A). Data obtained in the greenhouse at Manhattan on the reaction toward leaf rust shown by the Kanred and Fulcaster parents and the F_1 and F_2 generations of crosses between them, are given in Table VII.



Reaction of parents and F_2 segregates of a cross between Kanred and resistant Fulcaster to physiologic form 9 of leaf rust, when inoculated in the shooting stage

A.—Leaf of susceptible Kanred parent
B.—Leaf illustrating 93 F_2 segregates similar to Kanred in reaction
C.—Leaf illustrating 114 F_2 segregates intermediate in reaction
D.—Leaf illustrating 81 F_2 segregates similar to Fulcaster in reaction
E.—Leaf of resistant Fulcaster parent

TABLE VII.—*Reaction of the F_1 and F_2 progeny at three stages of development of parents and hybrids between Kanred and resistant and susceptible strains of Fulcaster, studied in the greenhouse at Manhattan, Kans.*

Parent or hybrid combination	Number of resistant, intermediate, and susceptible plants, when inoculated at various stages of development								
	Seedling stage [a]			Shooting stage			Heading stage		
	Resistant [b]	Intermediate [c]	Susceptible [d]	Resistant [b]	Intermediate [c]	Susceptible [d]	Resistant [b]	Intermediate [c]	Susceptible [d]
Resistant Fulcaster, 1922–23	140			140			140		
Susceptible Fulcaster, 1922–23			22			22			22
Kanred, 1922–23			62			62			62
F_1 Kanred×resistant Fulcaster, 1922–23 [e]		23			23			23	
F_1 Kanred×susceptible Fulcaster, 1922–23			40			40			40
Resistant Fulcaster, 1923–24	75			75			75		
Susceptible Fulcaster, 1923–24			32			32			32
Kanred, 1923–1924			36			36			36
F_2 Kanred×resistant Fulcaster, 1923–24 [e]	81	9	198	81	114	93	101	135	[f] 49
F_2 Kanred×susceptible Fulcaster, 1923–24	3		46	3		46	3		46
F_1 Kanred×resistant Fulcaster, 1923–24 [e]		6			6			6	

[a] Reaction of third to fourth leaves, not first seedling leaf.
[b] Showing a resistance of 0–1.
[c] Showing many normal uredinia, but also some flecking and necrosis, plants variable, 2–3.
[d] Mostly highly susceptible, 4.
[e] Includes reciprocal cross also, as behavior is the same.
[f] No record on three of the plants in the heading stage.

In the fourth or fifth-leaf seedling stage the F_1 plants of the crosses between Kanred and the resistant Fulcaster strains proved to be more or less susceptible. Some resistance, however, was present as evidenced by the numerous flecks and the smaller number and size of the uredinia, a susceptibility intermediate between classes 2 and 3. This indicates, therefore, a somewhat intermediate type of susceptibility for the seedling stage of the F_1. In crosses involving the moderately susceptible Fulcaster families 26 and 33, the F_1 plants were highly susceptible (class 4); and those of the cross between Kanred and susceptible Fulcaster were all very susceptible (class 4), with one exception which showed some signs of resistance.

In both the shooting and heading stages, the F_1 plants of the crosses between Kanred and susceptible Fulcaster, and between Kanred and Fulcaster families, 26 and 33 were highly susceptible (class 4), as were their parents also. The F_1 plants of the cross between Kanred and resistant Fulcaster were somewhat more resistant in the shooting and heading stages than in the seedling stage, as evidenced by the decrease in number of uredinia and an increase in flecking and necrosis, the reaction being about class 2 to 2+. They were still much more susceptible than the resistant Fulcaster parent at the same stages of development, being more nearly intermediate between the parents than in the seedling stage. One plant (No. 36) of this cross showed as high resistance as the resistant Fulcaster parent in all stages of development.

Greenhouse studies during the winter of 1923–24 were made with the same culture of leaf rust as that used on the F_1 generations in the previous winter, and it was still found to be largely form 9 with a

very slight mixture of form 3. The parental varieties, when inoculated, behaved in all stages as they had the previous winter. F_1 plants derived from new crosses between Kanred and susceptible Fulcaster and Kanred and resistant Fulcaster, made in the spring of 1923, also were studied, and they gave the same results (Table VII) as those obtained with F_1 plants of the same combinations the previous winter.

Results of Inoculating F_2 in the Seedling Stage

From the seed produced by the F_1 plants of Kanred by resistant Fulcaster (studied in 1922–23), 299 F_2 plants were grown in 4-inch pots in the greenhouse at Manhattan during the winter of 1923–24, and studied for reaction to leaf rust in three successive stages of development. Inoculation in the third-leaf or fourth-leaf seedling stage resulted in a marked segregation into two groups, one highly resistant (class 0–1) and the other highly susceptible (class 3–4). The family which descended from plant No. 36, noted above as resistant in the F_1, was markedly different from the others, producing 10 highly resistant plants and one susceptible. Judging from the action of this family in the F_1 and F_2, this was not a cross in 1922 but was self-pollinated. The presence of one susceptible plant in the F_2 is difficult to explain, but it may have been due to natural crossing of one flower with pollen from a susceptible plant in the spring of 1923, in which case the plant obtained would be susceptible. Eliminating this family from consideration, 288 plants remained from which dependable results were obtained. Of these, 207 were classified as susceptible or intermediate and 81 as resistant (Table VII). The ratio of susceptible to resistant plants is therefore 2.88:1.12. This gives a deviation of 0.12 ± 0.07, and D/PE equals 1.71, which is a very good fit.

The nearly complete absence of a class of individuals intermediate in their rust behavior in this stage of growth is in striking contrast to the results obtained in later inoculations. Only 9 out of the 288 plants in the seedling stage approximated an intermediate condition in rust behavior. The remainder fell definitely into resistant or susceptible classes. Apparently, therefore, the F_2 generation of this cross segregates definitely in the ratio of approximately three susceptible to one resistant individual in the seedling stage.

The behavior of the parental varieties and the F_1 plants toward leaf rust in all stages of growth was identical in the two years in which they were grown (Table VII).

Results of Inoculating F_2 in the Shooting Stage

After the hybrid and parental plants had been inoculated in the seedling stage no further inoculations were made until they reached the shooting or jointing stage. The intervening period is one of rather slow growth in which the plants exhibit a tendency toward dormancy such as is exhibited by most winter wheats when grown in the greenhouse. In the shooting stage, the wheat plant is making very rapid growth.

During the period between the seedling-stage and shooting-stage inoculations very little natural infection had occurred, and almost all of the seedling leaves which were infected at the time of the first

inoculation had died, leaving the plants nearly free from rust. This insured that none of the results obtained from the shooting-stage inoculation could be interpreted as being due to continued association of the host and parasite.

The rust material and methods used in this inoculation were the same as those employed in the seedling-stage inoculation. The rust was tested on a set of differential varieties, to make sure that no change had occurred in its strain content since it was last used. Notes were taken on the rust behavior of the plants about 14 days after inoculation.

The results obtained from inoculation of Kanred × resistant Fulcaster hybrids at this stage of growth differed somewhat from those expected on the basis of the earlier inoculations. A complicating factor was encountered in the appearance of numerous plants which exhibited an intermediate reaction to rust (see pl. 1). This condition was more marked than in any of the previous studies. The situation was further complicated by apparent differences in the degree of intermediacy exhibited by various plants. No attempt was made to separate the plants into phenotypic classes based on the type of intermediacy shown. To do so would have been very difficult, because of the intergradation of plants of this type. There was no difficulty in identifying the plants which had been classified as resistant in the seedling stage. All of these plants maintained their resistance, and most of them showed more nearly complete immunity than in the seedling stage. These plants were characterized by almost complete freedom from rust sori and an abundance of somewhat indistinct flecking (pl. 1, D and E).

It was the group classified as uniformly susceptible in the seedling stage which, in the shooting stage, contained plants that reacted as susceptibles and others that showed an intermediate type on infection. No definite ratio could be obtained from an analysis of the inoculation results in that class, because of the gradual change from the intermediate condition into complete susceptibility. Those plants definitely identifiable as intermediates were characterized by the presence of numerous large, sharp, yellow flecks accompanied by uredinia of varying size. There were usually, in addition, a number of minute uredinia surrounded by yellow necrotic areas and a few normal uredinia without accompanying killed areas. In the intermediate plants which most closely resembled the truly resistant or immune plants the upper leaves often had many large, sharply defined yellow flecks without uredinia, while the lower leaves showed few to many uredinia varying from minute to normal in size and accompanied by a moderate amount of flecking (see pl. 1).

As mentioned, the plants which had been classified as susceptible in the seedling stage could not be definitely classified in the shooting stage. On the basis of the classification as made, there were obtained 93 susceptibles (pl. 1, B and C), and 114 intermediates, a ratio of 1.35:1.65 instead of the expected 1:2. This condition very likely was due to the difficulty of distinguishing plants exhibiting a very slight degree of intermediacy from the completely susceptible individuals. In both of these classes the uredinia are large, normal, and very numerous. The intermediate plants exhibit a very slight yellowish flecking interspersed with uredinia, while the completely susceptible plants usually have no yellow areas. In cases of extremely

heavy infection, however, this distinction is not always clear because of the killing of the leaf tissues by the fungus, making correct classification extremely difficult and often impossible. Because of this difficulty these two classes will be considered together and will be classified as susceptible, although some of the readily recognizable intermediates show considerable resistance. If the practically immune hybrid and parental Fulcaster plants are to be classed as "resistant," all other plants fall naturally into a group with contrasted characters, which may be classified as "susceptibles."

Results of Inoculating the F_2 in the Heading Stage

All except three of the plants that were inoculated in the seedling and shooting stages were again inoculated as soon as they headed. The methods and the rust culture used were the same as those employed in the preceding inoculation. The results are given in Table VII.

The highly resistant F_2 segregates maintained their resistance in the heading stage. Many of the plants which had exhibited an intermediate type of reaction to rust in the shooting stage, however, approached true resistance in the heading stage. In many cases they could be distinguished from the truly resistant plants only by a sharper type of flecking and the presence of a small number of unedinia on the lower leaves. The truly resistant plants were characterized by a more indistinct type of flecking and almost complete absence of uredinia from all leaves. These facts demonstrate clearly that a change from complete susceptibility in the seedling to a very high type of resistance in the mature plant occurred in some of the plants. This behavior is in the direction of a reversal of dominance. The reason for this behavior is not clear, but the evidence indicates that those plants which are heterozygous for resistance are those in which this behavior takes place, while the homozygous resistant and susceptible plants maintain a constant reaction to leaf rust in all stages of growth.

The parental plants of resistant Fulcaster maintained the same high type of resistance in the heading stage which they had exhibited in the earlier stages of growth. Kanred, on the other hand, proved extremely susceptible in the heading stage, many plants carrying as high as 80 per cent of rust on the leaves.

The crosses between Kanred and the slightly resistant Fulcaster line 33, which in the F_1 were highly susceptible in the greenhouse, showed no segregation in the seedling stage in the F_2 generation, all 201 plants being highly susceptible (class 4). One family of the cross susceptible Fulcaster by Kanred, comprising 30 plants, also proved entirely susceptible in the F_2. The other family of this cross, consisting of 19 plants, gave 16 susceptible and 3 highly resistant in the F_2.

Results of Inoculating the F_3

During the winter of 1924–25 and the spring of 1925, some of the progenies from the F_2 plants of the Kanred by resistant Fulcaster were studied for their reaction in the seedling, shooting, and heading stages. The culture of rust used was the same as that employed in previous years. From studies made on this culture, however, it

apparently consisted entirely of form 9, the small proportion of form 3 having died. The data on F_3 inoculations are given in Table VIII.

TABLE VIII.—*Reaction of F_3 plants of the Kanred×resistant Fulcaster cross to leaf rust in the greenhouse, Manhattan, Kans., 1924–25*

Reaction of F_2 parent plant to leaf rust	Stage of growth when inoculated	Number of F_3 plants				
		Total	Immune	Resistant	Intermediate	Susceptible
Intermediate in one or more stages of growth	Seedling	60	8	6	25	21
	Shooting	56	18	13	10	15
	Heading	56	14	16	7	19
Seedling stage susceptible, shooting stage intermediate, heading stage resistant.	Seedling	97	2	18	49	28
	Shooting	94	22	33	10	29
	Heading	92	22	30	18	22
Resistant in all stages of growth	Seedling	383	254	120	6	3
	Shooting	375	348	21	5	1
	Heading	373	340	28	5	----
Susceptible in all stages of growth	Seedling	49	----	1	5	43
	Shooting	46	1	2	3	40
	Heading	42	1	1	3	37

SEEDLING-STAGE INOCULATIONS.—Five plants each of 77 F_3 families, derived from plants resistant at all stages of growth, were inoculated in the seedling stage. Of these families 70 bred true for resistance, six had only one plant each which was other than resistant, while one had two resistant and three intermediate plants. This latter family may be a case of true segregation, the F_2 plant having been incorrectly classified. The other six cases of variation from the expected, and possibly the seventh also, may have been due to cross pollination in the greenhouse the previous year, as it appears from evidence at hand that crossing occurs rather frequently in the greenhouse at Manhattan.

Six F_3 families of 10 plants each from F_2 plants which were classed as intermediate in their reaction to rust at one or more stages of growth, produced 14 resistant or immune, 25 intermediate, and 21 susceptible plants. This shows definite segregation approximating the expected 1:2:1 ratio. The deviation can be explained by the difficulty in distinguishing between susceptible and intermediate plants in all cases. With some plants the distinction is marked and the difference is very plain, and in other cases it is practically impossible to tell whether the plant is intermediate or susceptible. This undoubtedly is due in part to the rôle the physiology of the host and the environmental conditions play in the reaction of any plant to leaf rust.

Ten F_3 families from F_2 plants which were classified as susceptible in the seedling stage, intermediate in the shooting stage, and resistant in the heading stage produced 20 plants resistant or immune, 49 plants intermediate, and 28 susceptible. This indicates quite clearly that the F_2 parents were intermediates, and that the segregation in F_3 very closely approaches the expected ratio of 1:2:1. Here again the difficulty of distinguishing between susceptible and intermediate plants arose and is reflected in the figures. If the figures for both groups of intermediate plants are added, the ratio obtained is 34 resistant or immune: 74 intermediate : 49 susceptible. The expected would be 39:79:39, so this is a good fit for such small numbers.

Ten F_3 families of 5 plants each from F_2 plants which were susceptible in all stages of growth were inoculated. Six of these families bred true for susceptibility; 3 contained 4 susceptible, and 1 intermediate plants; and in 1 family there were 2 susceptible, 2 intermediate, and 1 resistant plants. The behavior of this latter family undoubtedly is due to a mistake in the classification of the F_2 plant from which it came. The occurrence of a single intermediate plant in each of the 3 otherwise susceptible families may be due to the difficulty of correctly classifying such plants or to the effect of the greenhouse conditions or of the physiology of the plant on its reaction to leaf rust. In general, it seems safe to state that the F_2 seedling classification was correct with but few exceptions.

SHOOTING-STAGE AND HEADING-STAGE INOCULATIONS.—When the F_3 plants were inoculated in the shooting stage there again was a noticeable change in the rust behavior of plants in segregating families. This change, like that which occurred in F_2, was manifested by a decrease in the number of susceptible plants and an increase in the number of resistants. This again caused an apparent change of dominance from susceptibility toward resistance. Many plants which were susceptible or intermediate in reaction in the seedling stage were resistant in the shooting stage.

The continuation of this change in the heading stage was not as marked in F_3 as in F_2, but in a number of cases it was evident. The data in Table VIII indicate, however, that the most marked change in rust behavior occurs at the shooting stage. The general tendency of plants to become more resistant as they grow older is shown not only in the behavior of plants in the segregating families, but also in the reaction of plants in homozygous resistant and susceptible families.

A few individuals in each of the supposedly homozygous groups did not breed true in any stage of growth (Table VIII). This may have been due to incorrect classification in F_2, or to natural crossing in the greenhouse the preceding season.

The F_3 results have shown that the only plants which can be classed as homozygous resistants are those either resistant or immune in all stages of growth. On this basis there are 117 susceptible (including susceptible and intermediate) and 31 resistant (including resistant and immune) plants in F_3 segregating families for which data are available in all stages of growth. The expected numbers, on the basis of segregation in a simple monohybrid ratio, would be 111 susceptible and 37 resistant plants. This gives a deviation of 6 ± 3.6.

Furthermore, the F_3 data show conclusively that only those individuals which are susceptible in all stages of growth are homozygous for susceptibility and can be expected to breed true. Those individuals which show marked changes in reaction to leaf rust at different stages of growth are intermediates and segregate in F_3, in ratios similar to those obtained in F_2. Two distinct types of intermediacy were noted: One in which the plants were susceptible in the seedling-stage, intermediate in the shooting stage, and resistant in the head ing stage; and another in which the lower inactive leaves were more or less susceptible, while the upper more active leaves showed only flecking, indicating resistance. The susceptible area moves up the

stem as the plants become older, but no cases were noted in which the entire plant became susceptible before maturity.

The F_3 results confirm the F_2 classifications with but few exceptions. It may be concluded, therefore, from the data obtained on this cross between Kanred and Fulcaster, that true resistance is a recessive character which is inherited in a manner indicating a single main-factor difference. However, the different types of intermediacy observed, and the evident increase in resistance as the plants of all classes grow older, possibly indicate the presence of modifying factors.

Hybrids With Malakoff as the Resistant Parent

Of the hybrids produced at Arlington Experiment Farm in 1921 with Malakoff as the resistant parent (Table III), three were grown in the F_1 generation in the greenhouse at La Fayette in the winter of 1921–22 and were studied for reaction to two physiologic forms of leaf rust. The results are given in Table IX. It is clear that the high resistance of Malakoff to form 1 is dominant in the F_1 of all three crosses. The F_1 of each hybrid was highly susceptible to form 6, as was to be expected, because in each case both parents were susceptible.

Table IX.—*Reaction of the parent varieties and F_1 generation of three crosses with Malakoff, C. I. 4898, to two physiologic forms of the leaf rust of wheat, Puccinia triticina*

Parents or hybrid combinations	Number of plants tested	Class of reaction to physiologic forms	
		No. 1	No. 6
Malakoff, C. I. 4898	10	0–1	4
Malakoff × Mammoth Amber	1	0–1	4
Mammoth Amber	10	4	4
Malakoff, C. I. 4898	11	0	4
Malakoff × Weissenberg	3	1	4
Weissenberg	8	4	4
Malakoff, C. I. 4898	10	0	4
Kanred × Malakoff	2	1	4
Malakoff × Kanred	25	1	4
Kanred	21	2+ to 3	3–4

During the winter of 1922–23 F_2 generations of nine of the hybrids were studied for reaction to form 12. The parent varieties reacted the same to this form as to form 1. This material included the seed obtained from the F_1 plants of the hybrids grown in the greenhouse in the previous winter as well as that obtained from some of the F_1 plants grown in the field nursery at Arlington Farm in 1922.

The contrast between the resistance shown by Malakoff and the susceptibility of the other parents was very marked (pl. 2), Malakoff showing only a slight flecking while the susceptible parents showed a heavy development of large uredinia. As in the previous cross, Kanred, which was used as the resistant parent for a number of hybrids studied in the field, is the susceptible parent in the Malakoff-Kanred cross in these greenhouses studies. As is shown by Table X, about one out of every four of the F_2 progenies was as susceptible as the susceptible parent. While the greater number of the remaining three-fourths were as highly resistant as the Malakoff parent (class 0),

several individuals in most of the families showed slightly less resistance (class 1). The latter group, however, was markedly resistant as contrasted with the susceptible parents and segregates. In Table XI all the plants showing a host reaction of either class 0 or 1 are grouped together as resistant.

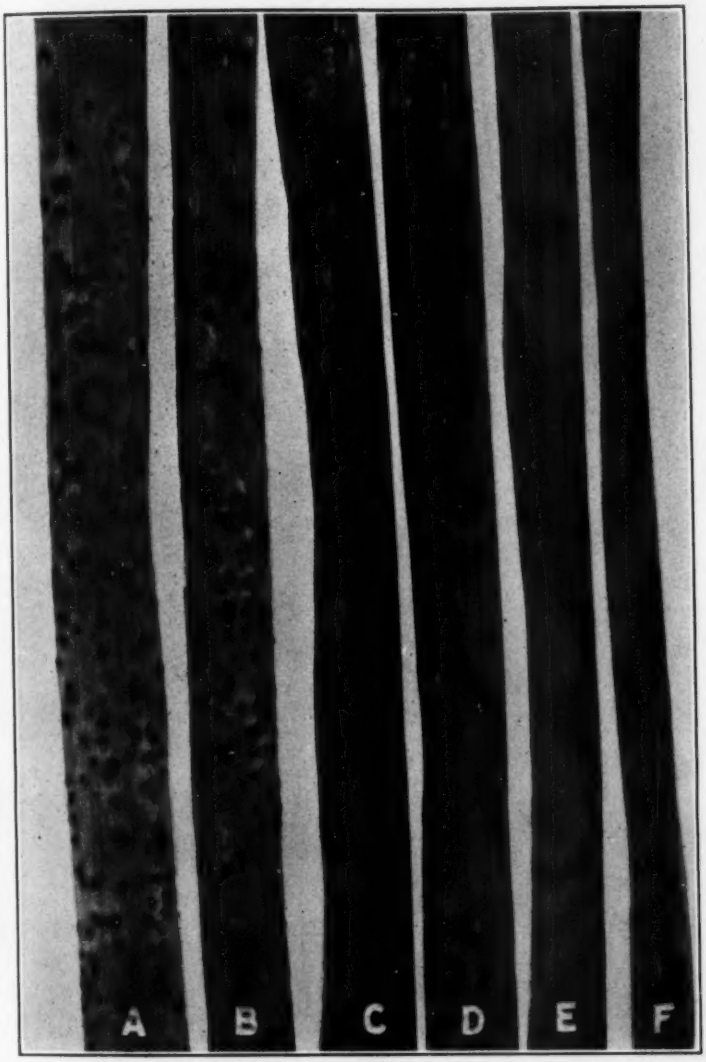
TABLE X.—*Reaction of the seedling stage of F_2 segregates of hybrids between Malakoff and different wheat varieties to physiologic form 12 of the leaf rust of wheat (Puccinia triticina) in the greenhouse at La Fayette, Ind.*

Malakoff crossed with—	Number of plants in classes of host reaction				
	Class 0	Class 1	Class 2	Class 3	Class 4
Mammoth Amber	67	43			37
Weissenberg	60	29			35
Kanred	108	41			43
Pennsylvania No. 44	146	3			50
Junior No. 6	124	64			74
Michigan Amber	40	20			30
Blue Ridge	125	31			65
Martin	119	1			41
Sol	115				43

It is evident from the data in Table XI that in all crosses except the one with Michigan Amber, segregation for resistance and susceptibility took place in a ratio very closely approximating 3:1. The greatest deviation from this ratio occurred in line 6 of the Malakoff × Michigan Amber cross, in which the deviation was 3.12 times the probable error. In line 7 of this same cross, however, the segregation in the F_2 was very close to a 3:1 ratio. The deviation was less than twice the probable error in all other crosses, except Malakoff × Blue Ridge, where it was 2.25 times the probable error. For the total F_2 plants the deviation was 2.67 times the probable error. Omitting line 6, the deviation is exactly twice the probable error.

All the F_2 plants were grown to maturity in the greenhouse. Seed from these was sown in the greenhouse bed during the winter of 1923–24, and the F_3 generation was studied for reaction to form 3, the parent varieties reacting the same to this from as to forms 1 and 12. Not all of the F_2 plants were represented, as several of them produced too few seeds to justify an F_3 study. This elimination was rather uniformly distributed, however, as is shown in Table XI.

As far as possible, the number of resistant and susceptible seedlings was determined in each F_3 family. However, toward the end of the study time was not available for such detailed counts, and families from susceptible F_2 plants of several hybrids and all the families of the hybrids with Kanred and Pennsylvania No. 44 were recorded only as uniform or nonuniform for resistance or susceptibility.



Reaction of parents and F_2 segregates of a cross between Malakoff, C. I. 4898, and Blue Ridge in the seedling stage in the greenhouse to physiologic form 12 of leaf rust

A.—Leaf of susceptible Blue Ridge parent
B.—Leaf illustrating 65 susceptible F_2 segregates
C, D, E.—Leaves illustrating 156 resistant F_2 segregates
F.—Leaf illustrating resistant Malakoff parent

TABLE XI.—*Reaction of the F_2 progeny and parents of hybrids between Malakoff and nine other varieties of common wheat to physiologic form 12, and of the F_3 progeny and parents to physiologic form 3, in the greenhouse at La Fayette, Ind. (segregation per 4 plants at ratio of 3 resistant to 1 susceptible).*

Parent or hybrid combination	F_2 generation and parents				F_3 generation and parents								
					Homozygous-resistant		Heterozygous			Homozygous-susceptible			
	Number of resistant plants [a]	Number of susceptible plants [b]	Deviation	Dev. / P. E.	Number of families	Number of plants	Number of families	Number of plants: Resistant	Number of plants: Susceptible	Number of families	Number of plants	Deviation [c]	Dev. / P. E.
Malakoff	195					895							
Malakoff×Mammoth Amber	110	37	0.01±0.10	0.10	38	909	72	1,476	452	34	1,130	0.06±0.10	0.60
	(2.99)	(1.01)			(1.06)		(2.00)			(0.94)			
Mammoth Amber		25									125		
Malakoff×Weissenberg	89	35	.13±.10	1.30	27	815	56	1,159	397	32	1,309	.11±.11	1.00
	(2.87)	(1.13)			(0.94)		(1.95)			(1.11)			
Weissenberg		25									95		
Malakoff×Kanred	149	43	.10±.08	1.25	41		86			40		.04±.09	.44
	(3.10)	(0.90)			(0.98)		(2.06)			(0.96)			
Kanred		23									150		
Malakoff×Pennsylvania No. 44	149	50	.01±.08	.12	44		89			45		.01±.09	.11
	(2.99)	(1.01)			(0.99)		(2.00)			(1.01)			
Pennsylvania No. 44		25									115		
Malakoff×Junior No. 6	188	74	.13±.07	1.86	36	434	71	694	236	43	1,146	.15±.10	1.50
	(2.87)	(1.13)			(0.96)		(1.89)			(1.15)			
Junior No. 6		25									112		
Malakoff×Michigan Amber:													
Line 6	29	18	.53±.17	3.12	9		12			16		.73±.19	3.84
	(2.47)	(1.53)			(0.97)		(1.30)			(1.73)			
Line 7	31	12	.12±.18	.67	9		8			13		.73±.21	3.48
	(2.88)	(1.12)			(1.20)		(1.07)			(1.73)			
Michigan Amber		26									55		
Malakoff×Blue Ridge	156	65	.18±.08	2.25	40	806	77	1,261	430	42	1,655	.06±.09	.67
	(2.82)	(1.18)			(1.01)		(1.94)			(1.06)			
Blue Ridge		25									129		
Malakoff×Martin	120	41	.02±.09	.22	36	686	77	1,279	424	36		.03±.10	.30
	(2.98)	(1.02)			(0.97)		(2.07)			(0.97)			

Martin		31								165		
Malakoff×Sol [d]	115 (2.91)	43 (1.09)	.09±.09	1.00	59 (1.06)	692	107 (1.93)	1,195	406	56 (1.01)		.01±.08	.12
Sol		27									125		
Total F_2 plants and F_3 families	1,136 (2.92)	418 (1.08)	.08±.03	2.67	339		655			357 (1.06)		.06±.03	2.00
Total F_2 plants (omitting line 6)	1,107 (2.94)	400 (1.06)	.06±.03	2.00	(2.94)								

[a] These plants were very highly resistant, belonging to classes 0–1.
[b] These plants were very susceptible, belonging to class 4.
[c] Deviation from expectation of 1 homozygous susceptible to 3 homozygous resistant and heterozygous families combined.
[d] In the F_3 generation 75 families were included, the parental F_2 plants of which were not studied.

From the data in Table XI it is apparent that a very close approximation to the expected ratio (one homozygous-resistant : two heterozygous : one homozygous-susceptible) was obtained. The only important exception was in the Malakoff × Michigan Amber cross, in which both lines 6 and 7 deviated rather widely from the expected ratio. The deviations in these lines, respectively, were 3.84 and 3.48 times their probable errors. As line 6 deviated more than three times its probable error in the F_2, it appears that the deviation in this cross is due to some unknown cause and not entirely to chance. Nearly all of the plants which were classified in the F_2 as susceptible to form 12 produced F_3 families in which all of the individuals were susceptible to form 3. Of the plants which were classified as resistant in the F_2 to form 12, approximately 1 out of 3 produced F_3 families which were pure for resistance to form 3. The remaining two-thirds produced families which had approximately 1 susceptible to 3 resistant individuals. In general, there were no marked differences between the group of F_2 individuals classified as 0 in resistance and those classified as 1. Both contained homozygous and heterozygous individuals in about the same proportion in the F_3.

A different physiologic form of leaf rust was used for each generation in these hybrids. This was necessary, as considerable difficulty was experienced in maintaining cultures of the rust over summer, and several, including forms 1 and 12, were lost. However, forms 1, 12, and 3, while differing in their action upon several varieties[8] behave virtually identically upon Malakoff. As the results of inoculation of F_3 families with form 3 agree so closely with the results obtained by inoculating the F_2 parents of those families with form 12, it seems quite certain that the resistance of Malakoff to these two forms is inherited as a unit character.

From these results on the F_2 and F_3 generations, it is apparent that the factor for resistance in Malakoff, C. I. 4898, to at least three physiologic forms of rust (forms 1, 3, and 12) is dominant in crosses with at least nine varieties of wheat. It is very probable that this also is true for its resistance to the three other forms (2, 4, and 11) to which this variety is known to be resistant. The resistance by Malakoff, C. I. 4898, to these physiologic forms is definitely shown to be due to a single factor.

Hybrids Between Varieties Differentiating Physiologic Forms of Leaf Rust

In the spring of 1921 and again in the spring of 1922 several crosses were made at La Fayette, Ind., using some of the varieties of wheat by which the various physiologic forms of leaf rust can be distinguished.[8] These crosses have been studied in several generations for reaction to several of the physiologic forms of leaf rust. These studies were made largely to obtain some preliminary information concerning the possibility of bringing together the resistance of two varieties to different physiologic forms of leaf rust, as Puttick (*18*) and Aamodt (*1*) have shown to be possible for stem rust of wheat.

[8] Mains, E. B., and Jackson, H. S. Physiologic specialization in the leaf rust of wheat, Puccinia triticina. Phytopathology 16: 89-120. 1926.

MALAKOFF, C. I. 4898×C. I. 3778

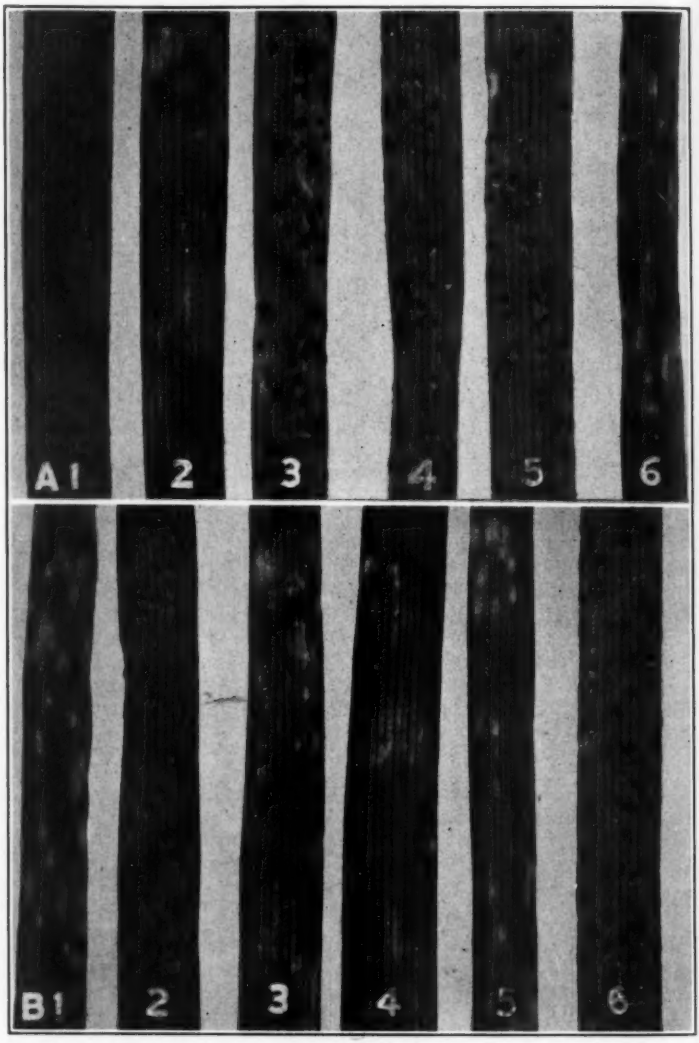
As already stated, Malakoff, C. I. 4898, is resistant to six physiologic forms of the leaf rust and susceptible to the other six. The variety C. I. 3778, while resistant to three of the physiologic forms to which Malakoff is resistant, is susceptible to three to which that variety is resistant. On the other hand, C. I. 3778 is resistant to three forms to which Malakoff is susceptible, and susceptible to three to which the latter also is susceptible.

In the winter of 1921–22 the F_1 generation was studied, in the seedling stage in the greenhouse, for reaction to a culture of physiologic form 5 of leaf rust. The parent Malakoff was highly susceptible (class 4) to this form. On the other hand, the parent C. I. 3778 was highly resistant (class 0–1). The F_1 plants were found to resemble most closely the Malakoff parent in their reaction, being classed as 3–4. The F_1 plants were all grown to maturity in the greenhouse.

During the winter of 1922–23 the F_2 generation, consisting of 85 plants, was studied in the greenhouse in the seedling stage for reaction to two cultures of leaf rust belonging to two distinct physiologic forms. The culture first used belonged to form 12. To this form Malakoff, C. I. 4898, is highly resistant (class 0–1) and C. I. 3778 is highly susceptible (class 4). (Pl. 3, B 1 and 2.) It is to be noted that the reaction to form 12 shown by these varieties is the reverse of the reaction shown to form 5 (pl. 3, A 1 and 2). As shown in Table XII, of the 85 F_2 plants inoculated with form 12, 65 were resistant, of which 18 were somewhat less resistant than the Malakoff parent (pl. 3 B). On the other hand, 20 were as highly susceptible as the susceptible parent, C. I. 3778. This is a very close approximation to a 3 : 1 ratio, with resistance dominant, indicating that the resistance of Malakoff to form 12 is inherited in this hybrid in the same manner as in the nine Malakoff hybrids discussed above (Table XI).

TABLE XII.—*Reaction of the F_2 progeny of three wheat hybrids to physiologic forms 5 and 12 of leaf rust, the parents being differential hosts for these forms in the greenhouse at La Fayette, Ind.*

Parent or hybrid combination	Physiologic form	Number of plants in reaction classes					Total resistant	Total susceptible	Segregation per 4 plants	Deviation	Dev. / P. E.
		Class 0	Class 1	Class 2	Class 3	Class 4					
Malakoff, C. I. 4898 × C. I. 3778	5	12	8	1	.....	64	21	64	0.99:3.01	0.01±0.13	0.08
	12	17	30	18	.....	20	65	20	3.06:0.94	.06+ .13	.46
Malakoff, C. I. 4898	5	.....	.....	.....	.....	25	.....	25	.....	.....	.....
	12	22	.....	.....	.....	.....	22	.....	.....	.....	.....
C. I. 3778	5	.....	15	.....	.....	.....	15	.....	.....	.....	.....
	12	.....	.....	.....	.....	18	.....	18	.....	.....	.....
Malakoff, C. I. 4898 × Webster, C. I. 3780	5	66	.....	.....	65	88	66	153	1.21:2.79	.21± .08	2.63
Malakoff, C. I. 4898	5	.....	.....	.....	.....	15	.....	15	.....	.....	.....
Webster, C. I. 3780	5	30	.....	.....	.....	.....	30	.....	.....	.....	.....
Norka, C. I. 4377 × C. I. 3756	5	20	8	8	.....	13	36	13	2.94:1.06	.06± .17	.35
	12	28	3	4	.....	13	35	13	2.92:1.08	.08± .17	.47
Norka, C. I. 4377	5	.....	.....	.....	.....	24	.....	24	.....	.....	.....
	12	25	.....	.....	.....	.....	25	.....	.....	.....	.....
C. I. 3756	5	30	.....	.....	.....	.....	30	.....	.....	.....	.....
	12	.....	.....	.....	.....	26	.....	26	.....	.....	.....

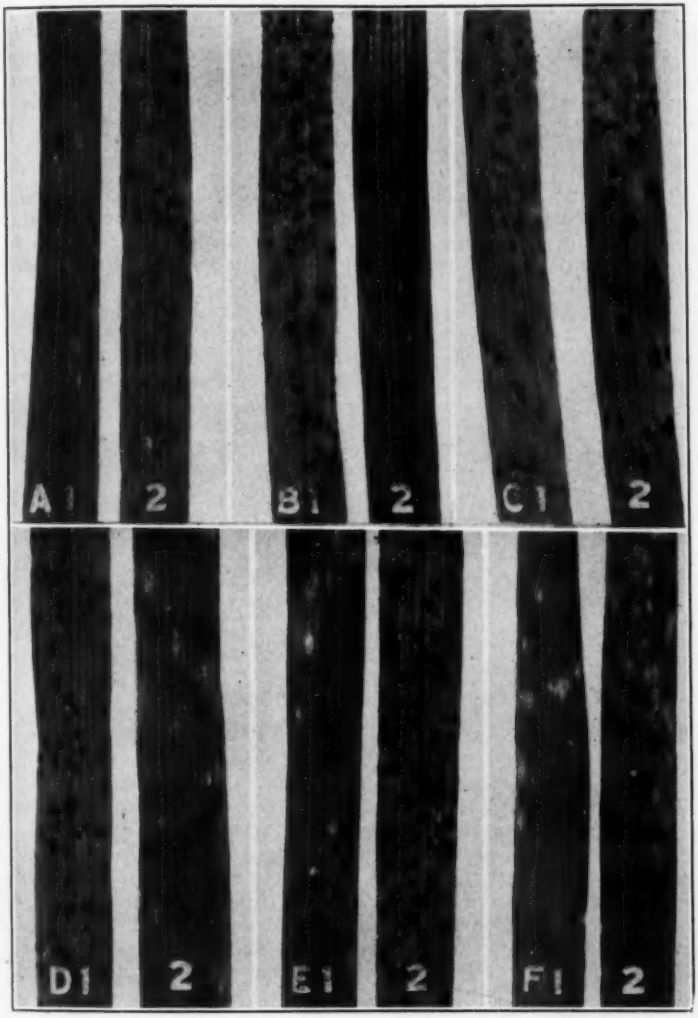


Reaction of a cross between Malakoff, C. I. 4898, and C. I. 3778 in the seedling stage in the greenhouse to physiologic forms 5 and 12 of leaf rust

A.—Reaction to form 5: (1) Leaf of susceptible Malakoff parent; (2) leaf of resistant C. I. 3778 parent; (3, 4, and 5) leaves illustrating 64 susceptible F_2 segregates; (6) leaf illustrating 21 resistant F_2 segregates

B.—Reaction to form 12: (1) Leaf of resistant Malakoff parent; (2) leaf of susceptible C. I. 3778 parent; (3, 4, and 5) leaves illustrating 65 F_2 resistant segregates; (6) leaf illustrating 20 F_2 susceptible segregates

(Note that the resistance of C. I. 3778 to form 5 is recessive, while the resistance of Malakoff to form 12 is dominant)



Combinations of resistance and suceptibility to physiologic forms 12 and 5 of leaf rust shown by parents and F_2 segregates of a cross between Malakoff, C. I. 4898, and C. I. 3778

A.—Leaves of Malakoff parent, illustrating: (1) Resistance to form 12, and (2) susceptibility to form 5

B.—Leaves of C. I. 3778 parent, illustrating: (1) Susceptibility to form 12, and (2) resistance to form 5

C.—Leaves illustrating reaction of 15 F_2 segregates: (1) Susceptible to form 12, and (2) susceptible to form 5

D.—Leaves illustrating reaction of 5 F_2 segregates: (1) Susceptible to form 12, and (2) resistant to form 5

E.—Leaves illustrating reaction of 49 F_2 segregates: (1) Resistant to form 12, and (2) susceptible to form 5

F.—Leaves illustrating reaction of 16 F_2 segregates: (1) Resistant to form 12, and (2) resistant to form 5

The infected leaves were trimmed from these 85 plants, and the plants were then inoculated with a culture of form 5. The reaction of the parents to this form is reversed, Malakoff being susceptible and C. I. 3778 resistant. The reaction of the 85 F_2 plants apparently was almost exactly reversed (Table XII), only 21 plants being resistant while 64 were susceptible, which is an almost exact 3:1 ratio, but with resistance recessive in this case instead of dominant as before (pl. 3, A).

However, when the notes for the reaction of each plant to both of the forms of rust are examined, it is found that the reaction in all cases is not reversed, but that the plants can be grouped into four classes according to the reaction shown to the two forms, as in Table XIII and Plate 4. The numbers of individuals in these groups approximate the ratio 1:3:3:9, indicating that the resistance of Malakoff to form 12 and the resistance of C. I. 3778 to form 5 are each dependent on a single independently inherited factor. It is also to be noted that 16 plants of the F_2 population were resistant to both the forms of rust, thus combining the resistance of the two parents.

TABLE XIII.—*Number of F_2 plants of two wheat hybrids showing combinations of susceptibility and resistance to physiologic forms 5 and 12 of leaf rust*

Hybrid combination	R. to F. 5 R. to F. 12	R. to F. 5 S. to F. 12	S. to F. 5 R. to F. 12	S. to F. 5 S. to F. 12	Expected segregation on 9:3:3:1 basis	χ^2	P
Malakoff × C. I. 3778	16	5	49	15	16:5:48:16	0.083	Very close fit.
Norka × C. I. 3756	27	9	9	4	28:9:9:3	.369	Do.

R = Resistant. S = Susceptible. F = Physiologic form.

The F_2 plants were grown to maturity, and during the winters of 1923–24 and 1924–25 the F_3 progenies were studied in all cases where enough seed was produced to make such study worth while. Only the progenies of F_2 plants resistant to both physiologic forms were studied during the winter of 1923–24. During 1924–25 the rest of the F_3 families were studied. The method employed was to inoculate the plants first with physiologic form 5. All families which were pure for resistance to form 5 were then inoculated with form 11, form 12 not being available. Form 11, while differing from form 12 upon some varieties, was similar in its behavior upon Malakoff and C. I. 3778. This method reduced considerably the families studied for susceptibility to form 11, but concentrated the study upon the families homozygous for the desired character, resistance.

Of the 85 F_2 plants, only 56 produced enough seed to justify study in the F_3. Of these, 38 had been susceptible and 18 resistant to form 5 in the F_2. Of the 38 F_3 families from susceptible F_2 plants, 15 bred true for susceptibility, a total of 363 plants proving to be susceptible. Twenty-three families from susceptible F_2 plants were found to be segregating, producing a total of 520 susceptible and 164 resistant plants, or a ratio fairly close to 3:1. The 18 F_3 families from resistant F_2 plants all bred true for resistance, a total of 679 plants proving to be resistant to form 5.

The 18 resistant F_3 families were from F_2 plants, of which 3 were susceptible and 15 resistant to form 12. When inoculated with physiologic form 11, the 3 families from susceptible F_2 plants, involving a total of 156 plants, all were pure for susceptibility. Of the remaining 15 families from resistant F_2 plants, 8 proved to be heterozygous, 68 individuals being susceptible and 205 resistant, a ratio very close to 1:3. Seven families involving 245 plants bred pure for resistance to form 11, and therefore were pure for resistance to both forms of leaf rust.

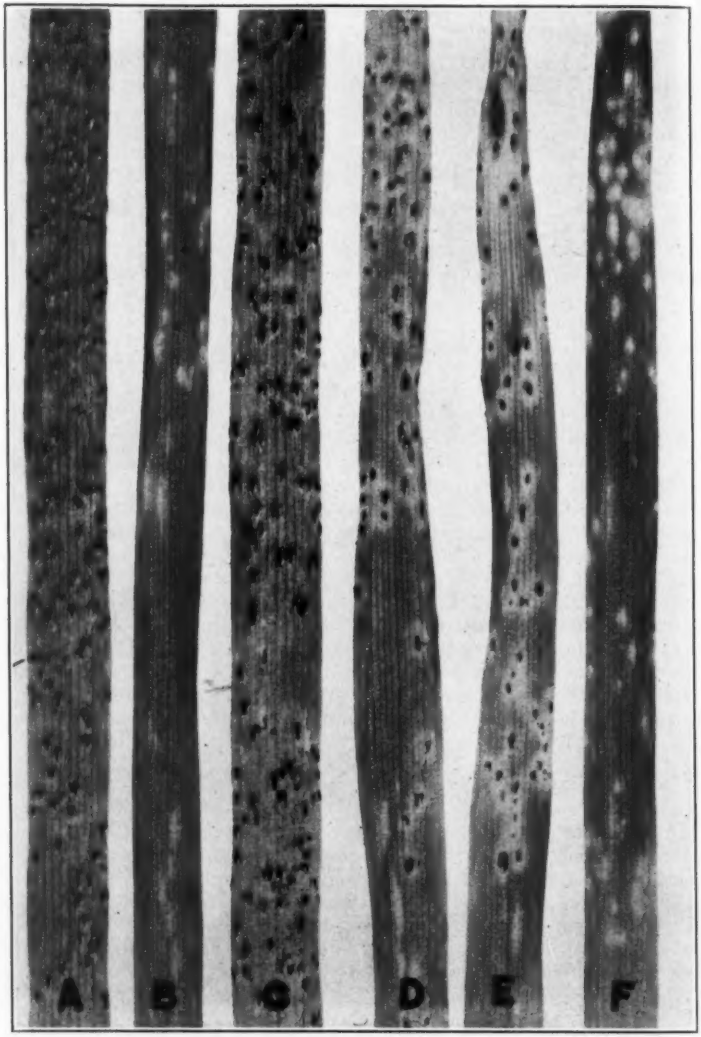
The ratio of homozygous and heterozygous families in the F_3 generation deviated somewhat from the expected 1:2:1 ratio. For reaction to form 5, the ratio was 15 homozygous-susceptible: 23 heterozygous : 18 homozygous-resistant. For reaction to form 11, the ratio was 3 homozygous-susceptible : 8 heterozygous : 7 homozygous-resistant. This deviation from the expected ratio is not regarded as significant, as the number of families concerned is small. The segregation of the heterozygous families to both forms of rust approaches very closely a ratio of 3 : 1, susceptibility to form 5 being dominant and resistance to form 11 being dominant. In other words, the type of reaction shown by Malakoff to the two forms was dominant in both cases.

The most important point, however, is that seven F_3 families combined the resistance of the parents, namely, Malakoff to form 11 and C. I. 3778 to form 5. Still further evidence of this was furnished by a study of F_4 progenies of these lines. During the winter of 1924–25, 712 F_4 plants obtained from 47 of the F_3 plants resistant to both forms 5 and 11, derived from 5 of the F_2 plants resistant to both physiologic forms 5 and 12, were inoculated first with form 5 and then with form 11. All 712 plants proved to be resistant to both forms. These lines therefore not only combine the resistance of the two parents, but are breeding true for resistance to both form 5 and form 11.

Malakoff, C. I. 4898×Webster, C. I. 3780

The variety Webster, C. I. 3780, is resistant not only to the six physiologic forms to which Malakoff is resistant, but shows high resistance to two forms to which Malakoff is susceptible. The F_1 plants of the cross between these two varieties were inoculated with a culture of form 5, to which Malakoff is highly susceptible (class 4) and C. I. 3780 is highly resistant (class 0). The F_1 plants inclined toward intermediacy, the reaction class being 2+ or 3. Although not as susceptible as Malakoff, they nevertheless were much closer to that variety than to the resistant Webster, C. I. 3780.

From seed of these F_1 plants, 219 F_2 plants were grown in the greenhouse during the winter of 1922–23. These were inoculated with the same culture, form 5, as was used on the F_1 plants the previous winter, with the result shown in Table XII and Plate 5. While 66 plants were as highly resistant as the Webster parent and 88 as susceptible as Malakoff, 65 resembled the F_1 plants in being somewhat less susceptible than Malakoff. This is not close to the 1 : 2 : 1 ratio expected in true intermediacy. However, part of this discrepancy doubtless is due to difficulty in classifying some of the segregates. Considering plants of classes 3 and 4 as susceptible, because



Reaction to physiologic form 5 of parents and F_2 segregates of a cross between Malakoff, C. I. 4898, and Webster, C. I. 3780, in the seedling stage in the greenhouse

A.—Leaf illustrating susceptibility of Malakoff parent
B.—Leaf illustrating resistance of Webster parent
C.—Leaf illustrating high susceptibility (similar to that of Malakoff) of 88 F_2 segregates
D, E.—Leaves illustrating a susceptibility slightly less than that of Malakoff shown by 65 F_2 segregates.
F.—Leaf showing high resistance (similar to that of Webster) of 66 F_2 segregates

they generally are so classed in comparison with those of classes 0, 1, and 2, a segregation is obtained in this hybrid of 66 resistant to 153 more or less susceptible plants, or a fairly close approximation to a 1 : 3 ratio, the deviation being 0.21 ± 0.08, or 2.63 times the probable error.

F_3 plants from seed of 40 of the 66 resistant F_2 plants were grown in the greenhouse during the winter of 1923–24 and inoculated with the same culture of leaf rust used on the F_1 and F_2 generations. All of the 658 plants of these 40 families showed a resistance similar to that of Webster, C. I. 3780. Furthermore, as it seemed desirable to know whether plants classed in the F_2 as intermediate in their rust reaction actually were heterozygous, seed from 16 of the F_2 susceptible (class 4) and 14 of the F_2 intermediate (class 3) plants was sown and F_3 plants grown in the greenhouse during the winter of 1924–25, those F_2 plants being selected which supplied enough seed for a thorough test. The F_3 plants thus obtained were inoculated with the form 5 used in previous years. Of the F_3 families from F_2 plants classified as class 4 in host reaction, 2 showed segregation and the remaining 14 all bred true for high susceptibility. On the other hand, of the F_3 families from F_2 plants classified as intermediate (class 3), 2 produced only susceptible plants while all of the remaining 12 showed segregation. The 14 families showing segregation produced a total of 165 susceptible (class 4) plants, 342 intermediate (class 2+ or 3) and 170 resistant (class 0). This is a very close approximation to a 1:2:1 ratio. It thus appears that plants of class 3 reaction in the F_2 were more often heterozygous than those of class 4. It also appears that, as a rule, in the study of the F_2 generation, only those fairly susceptible plants showing the most pronounced deviation from the susceptible parent were classed as intermediate (class 3).

During the winter of 1924–25, 605 F_4 plants derived from 19 of the F_3 families which were pure for resistance the previous year, were inoculated with form 5 and all were found to be resistant. These lines therefore are homozygous for resistance to form 5.

Apparently the resistance of Webster, C. I. 3780, and the susceptibility of Malakoff to form 5 are due to a single main allelomorphic pair of factors. There is no such marked dominance shown as in other hybrids. Heterozygous individuals, although approaching the susceptible Malakoff parent in reaction, are still somewhat less susceptible and may be distinguished from it fairly accurately by close study and comparison with the susceptible and resistant parents.

Norka, C. I. 4377 × C. I. 3756

Only two seeds were obtained in this cross, and the two F_1 plants were grown in the greenhouse during the winter of 1921–22. The seed from all these was sown in the greenhouse in the fall of 1922, and the seedlings were inoculated successively with forms 12 and 5. As shown in Table XII, Norka is highly resistant to form 12 and highly susceptible to form 5. On the other hand, C. I. 3756 is highly resistant to form 5 and highly susceptible to form 12. One family proved to be not a cross, all 31 plants being highly susceptible to form 5 and highly resistant to form 12, indicating that the Norka

parent in this instance had been self-fertilized. The other family, comprising 49 plants, behaved as shown in Table XII. Apparently the resistance of Norka to form 12 and the resistance of C. I. 3756 to form 5 are dominant in this hybrid, and the grouping of the individuals according to their reaction to both forms approaches a 1:3:3:9 ratio (Table XIII), indicating that the resistance of each parent is due to a single factor, each of which is inherited independently.

During the winters of 1923–24 and 1924–25 the F_3 generation was studied for reaction to physiologic form 5. Those F_3 families from F_2 plants resistant to both forms were studied in the first winter, and the rest were studied in the second winter so far as sufficient seed was available. The culture of form 12 was lost during the summer of 1923, and no other suitable culture was available at the time to take its place. The study with form 5, however, has shown a definite inheritance of the resistance of C. I. 3756.

Of the 48 F_2 plants, 33 produced enough seed to justify study in the F_3 generation. Six of these F_3 families were from F_2 plants classed as susceptible. These all bred true for susceptibility in the F_3, a total of 162 plants showing a high susceptibility to form 5. The remaining 28 families were from plants classed as resistant in the F_2 generation. Of these, 16 families proved to have been derived from heterozygous F_2 plants, 176 plants being highly susceptible and 515 resistant, or very near a 1:3 ratio and agreeing with the F_2 segregation. Eleven F_3 families from F_2 resistant plants bred true for resistance, a total of 453 plants being resistant.

During the winter of 1924–25 F_4 plants derived from some of those plants resistant to both form 5 and form 12 in the F_2, and those F_3 families which bred true for resistance to form 5, were studied for reaction to form 5. This involved 1,073 F_4 plants derived from 108 F_3 plants belonging to 8 F_2 families. These all proved to be resistant to form 5. The reaction of these lines to form 12 still remains to be studied, but there is no reason to believe that among them there will not be found lines homozygous for resistance to both forms 5 and 12.

The behavior of some of these lines in the field nursery at La Fayette during the summer of 1924 is of interest. Both parents, Norka and C. I. 3756, were heavily rusted. Certain of the lines derived from the hybrid between these two varieties were only slightly rusted. Studies of collections of the rust from this nursery now being made indicate that several physiologic forms of rust were present, to which the varieties Norka and C. I. 3756 were reciprocally resistant and susceptible. For this reason they both showed high susceptibility in the nursery. Some of the segregates from the hybrid, however, combined the resistance of the two varieties and they were resistant while both parents seemed to be highly susceptible.

DISCUSSION

The results in this study show that resistance to leaf rust often is inherited in a very definite manner. Several critical experiments seem to prove that the inheritance observed is Mendelian in character. In some of the experiments, however, the evidence is obscured by the complicating effect of the environment. Inasmuch as an important object of these investigations was the development of commercial

strains of wheat combining the leaf-rust resistance of certain varieties, and the adaptation and other good qualities of other varieties, most of the experiments have been conducted in the field nurseries. The various intergrading degrees of resistance and susceptibility, complicated by varying conditions of soil and climate, time of maturity and mixture of physiologic forms of rust, have made an accurate determination of the facts of inheritance difficult, if not impossible, in field-grown material.

It is apparent, however, in the material grown in the field, that resistance to leaf rust is inherited. The F_1 of crosses in which Kanred was used as the resistant parent usually equaled or approached the susceptibility of the susceptible parent. In the F_1 of the crosses in which Malakoff was used as the resistant parent, intermediacy in reaction was the usual condition, with sometimes a dominance of resistance. In the F_2 of both series of crosses a small proportion of the plants either equaled or exceeded the resistance of the resistant parents. However, these did not all breed true in the F_3 generation, but a number of the families and several selections from them apparently were fairly uniform for rust resistance in the F_4. In these resistance was combined with many of the plant characters of the susceptible parent.

The resistance of Kanred is not yet thoroughly understood. It has shown more resistance in some localities than in others, and has varied in its reaction in different years at the same place. It has been very susceptible in the seedling stage in the greenhouse at Manhattan, yet considerably resistant in the fully developed stage in general field culture. It is possible that more than one genetic factor is concerned in the resistance of this variety.

The results obtained in the studies carried out in the greenhouses at La Fayette and Manhattan are more definite than the field results. For the most part, these studies were made with pure physiologic forms of the leaf rust. In all of the hybrids studied, resistance to physiologic forms of leaf rust was inherited in a very definite manner. At least in the early stages of development of the wheat plants in each hybrid progeny, resistance and susceptibility apparently are dependent on a single allelomorphic pair of factors. In the hybrids where Malakoff is the resistant parent, resistance is dominant. In the hybrid between Malakoff and Webster, C. I. 3780, heterozygous plants show a certain intermediate condition, being less susceptible than the susceptible parent, although nearer to this than to the resistant parent. With Fulcaster as the resistant parent in crosses with Kanred, susceptibility was dominant in the early stages of F_2. In later stages of development a majority of the susceptible plants become more or less resistant. As these plants which changed in their reaction, when tested in the F_3 for the most part proved to be heterozygous, and as the F_1 plants also showed somewhat more resistance in later than in earlier stages of development, apparently the interesting situation occurs that heterozygous plants in this cross are indistinguishable in reaction in the young stages from homozygous susceptibles, while in later stages of development they usually can be distinguished by more or less resistance. It may be that other factors are involved, possibly derived from the Kanred parent, although the latter in this particular study showed no evidence of any such resistance.

In some of those hybrids in which the parents are reciprocally resistant and susceptible to two physiologic forms of leaf rust, the interesting fact obtains that resistance to one physiologic form is dominant and resistance to the other is recessive. Also, in such hybrids the resistance and susceptibility shown by the two parents to the two physiologic forms are inherited as two independent pairs of allelomorphic factors. In consequence, the resistance of the two parents, each to a different physiologic form, has been combined in some of the segregates which have been found resistant to both forms. It is probable that the resistance of these parents to other physiologic forms also is combined in these segregates. These have not been sufficiently studied yet to make certain of this point. The behavior of a number of the Malakoff hybrids to several different physiologic forms to which Malakoff is resistant suggests such conclusions.

As far as these studies have gone, the results are similar to those obtained in investigations with other rusts of wheat. In studies of the inheritance of resistance to the stem rust of wheat (*Puccinia graminis*) Clark (*6*) recently found that resistance in a cross between Kota and Hard Federation was recessive and occurred in the F_2 generation in about a 1:15 ratio. No F_3 family derived from these F_2 plants bred true. Aamodt (*2*) found that in a hybrid between Kanred and Marquis wheats the resistance of Kanred to physiologic form 1 of stem rust was inherited as a simple factor or as several linked factors with resistance dominant, a ratio of three resistant to one susceptible being produced in the F_2. Segregates homozygous for resistance to this form were also resistant to seven other physiologic forms to which Kanred is resistant. Melchers and Parker (*14*) obtained similar results with the same cross. Hayes and Aamodt (*11*) studied a cross between Kota and Marquis for resistance to two physiologic forms of stem rust. They found that resistance to both forms was combined in 3 out of 372 F_3 families. To form 27, Kota was immune and Marquis resistant. Besides homozygous immune and resistant families in the F_3 generation, families homozygous for susceptiblity were obtained. Four types of heterozygous families were obtained. Immunity was dominant to both resistance and susceptibility. The results are explained on the basis of two independently inherited factors for immunity and resistance contained in the Kota and Marquis parents, respectively, each factor being allelomorphic and dominant to a factor for susceptibility.

Puttick (*18*) found that in a cross between Mindum (a durum variety) and Marquis (a common variety), reciprocally resistant and susceptible to physiologic forms 1 and 19 of stem rust, 35 F_2 plants out of a total of 388 combined the resistance of both parents. All gradations between complete susceptibility and immunity occurred. It is considered that there is some evidence of a single main pair of genetic factors for the reaction to form 19. Harrington and Aamodt (*9*), in crosses between durum varieties, obtained somewhat similar results combining resistance to forms 1 and 34 of stem rust. Hayes, Parker, and Kurtzweil (*10*) found that resistance to a physiologic form of stem rust was partially dominant in an emmer-common cross, while it was recessive in a durum-common cross. There was some linkage of resistance with durum and emmer characters. Segregates were

obtained which were even more resistant than the durum parent. Waldron (*21*) obtained somewhat similar results in a durum-common cross. Interpretation of the results is complicated by the sterility which occurs in such hybrids. Except in the last two investigations, no evidence of linkage of rust resistance was obtained.

In his investigation of the inheritance of resistance of wheat to the stripe rust (*Puccinia glumarum*), Biffen (*4*) found that suceptibility was dominant and that the ratio in the F_2 was 3 susceptible plants to 1 resistant. Nilsson-Ehle (*16*) using different parent varieties, did not find such clear-cut dominance or segregation, but did observe transgressive segregation. He considered multiple factors necessary to the explanation of his results. Armstrong (*3*) obtained results concerning the inheritance of resistance to this rust closely approximating those of Biffen.

It therefore is apparent that, as with the other rusts of wheat, resistance to leaf rust varies in the manner in which it is inherited. It seems evident that what is being called leaf-rust resistance is a more or less similar manifestation of a number of different factors of marked physiologic difference. Whatever these differences may be, it is evident that they can be brought together, and it seems possible that strains of wheat may be developed in which these various factors will be combined and thus provide a wheat resistant to all the physiologic forms of leaf rust.

SUMMARY

The inheritance of resistance to the leaf rust of wheat (*Puccinia triticina*) was studied in crosses between resistant and susceptible varieties. In crosses with Kanred as the resistant parent and each of several susceptible varieties as the other parent, studied in field nurseries at Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., Knoxville, Tenn., and La Fayette, Ind., the F_1 generation was either as susceptible as the susceptible parent or to some degree intermediate. In the F_2 there were intergrading degrees of susceptibility, but with some segregates showing the resistance of Kanred. Many of these segregates produced susceptible plants in the F_3 generation, but others were resistant. In the F_4 several lines were obtained apparently pure for resistance and possessing desirable characters of the susceptible parent. The resistance of Kanred may depend on several factors.

In 11 crosses with Malakoff as the resistant parent, and various susceptible varieties as the other parent, studied in field nurseries at Arlington Experiment Farm, the F_1 sometimes was intermediate in resistance, and sometimes resistance was dominant. In later generations, the resistance of Malakoff was inherited but, on account of a mixture of physiologic forms of leaf rust in the field, it is not possible at present to draw any definite conclusions concerning the factors involved.

A study of nine of these crosses with Malakoff, in the seedling stage in the greenhouse at La Fayette, in which pure cultures of physiologic forms of leaf rust were used, has demonstrated that the resistance of Malakoff to certain of these physiologic forms is dependent on a single dominant genetic factor. Approximately three

resistant to one susceptible segregates appeared in the F_2 generation. In the F_3 generation, the susceptible segregates bred true, while only one out of three of the resistant bred true, the other two segregating again into three resistant and one susceptible.

In a cross between Malakoff as the susceptible parent, and Webster, C. I. 3780 as the resistant parent, studied for reaction to physiologic form 5 in the seedling stage in the greenhouse, heterozygous F_2 plants were somewhat intermediate in susceptibility but approached Malakoff so closely in some cases that they were difficult to distinguish from homozygous-susceptible plants. Apparently resistance is dependent upon a single main-factor difference, a segregation of 1 resistant : 2 intermediate : 1 susceptible occurring.

A cross having a selection of Fulcaster resistant to physiologic form 9 for the resistant parent and Kanred as the susceptible parent was studied in the greenhouse at Manhattan, Kans., in the seedling, shooting, and heading stages. In the seedling stage the F_1 plants were somewhat more resistant than the susceptible parent in its reaction to form 9, while in the shooting and heading stages they were more nearly intermediate in reaction in comparison with the resistant and susceptible parents. Heterozygous plants in the F_2 became progressively more resistant in successive stages of development, while homozygous-resistant and homozygous-susceptible plants maintained resistance and susceptibility, respectively, in all stages. Apparently resistance in this hybrid is dependent for its expression on a single main-factor difference.

In the F_2 of a cross between Malakoff and C. I. 3778, studied in the seedling stage for reaction to forms 5 and 12, Malakoff being susceptible to form 5 and resistant to form 12, and C. I. 3778 showing the reverse action, the resistances of the two parents were independently inherited. The resistance of Malakoff to form 12 was dominant, while the resistance of C. I. 3778 to form 5 was recessive. In the F_2 generation segregation was obtained approximately in the ratio of 1 plant resistant to form 5 and susceptible to form 12 : 3 plants resistant to both forms : 3 plants susceptible to both forms : 9 plants susceptible to form 5 and resistant to form 12. This indicates that the resistance of each parent is dependent on a single independently inherited factor. Similar results were obtained in a study of the inheritance of the resistance of Norka and C. I. 3756 to forms 5 and 12, with the exception that as the resistance of Norka to form 12 and of C. I. 3756 to form 5 was dominant, the ratio in the F_2 was 1 plant susceptible to both forms : 3 plants susceptible to form 5 and resistant to form 12 : 3 plants resistant to form 5 and susceptible to form 12 : 9 plants resistant to both forms.

Resistance to the various physiologic forms of leaf rust is due therefore to different factors, or groups of factors inherited as a unit, the different factors or groups being independently inherited. These may be brought together, thus uniting in a single strain the resistance to the various physiologic forms possessed by different varieties.

LITERATURE CITED

(1) AAMODT, O. S.
1922. THE INHERITANCE OF RESISTANCE TO SEVERAL BIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI IN A CROSS BETWEEN KANRED AND MARQUIS WHEATS. (Abstract) Phytopathology 12: 32.

(2) ———
1923. THE INHERITANCE OF GROWTH HABIT AND RESISTANCE TO STEM RUST IN A CROSS BETWEEN TWO VARIETIES OF COMMON WHEAT. Jour. Agr. Research 24: 457–470, illus.

(3) ARMSTRONG, S. F.
1922. THE MENDELIAN INHERITANCE OF SUSCEPTIBILITY AND RESISTANCE TO YELLOW RUST (PUCCINIA GLUMARUM, ERIKSS. ET HENN.) IN WHEAT. Jour. Agr. Sci. 12: 57–96.

(4) BIFFEN, R. H.
1907. STUDIES IN THE INHERITANCE OF DISEASE-RESISTANCE. Jour. Agr. Sci. 2: 109–128.

(5) CARLETON, M. A.
1899. CEREAL RUSTS OF THE UNITED STATES. U. S. Dept. Agr., Div. Veg. Physiol. and Path. Bul. 16, 74 p., illus.

(6) CLARK, J. A.
1925. SEGREGATION AND CORRELATED INHERITANCE IN CROSSES BETWEEN KOTA AND HARD FEDERATION WHEATS FOR RUST AND DROUGHT RESISTANCE. Jour. Agr. Research (1924) 29: 1–47, illus.

(7) DIETZ, S. M.
1925. THE INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS AVENAE. (Abstract) Phytopathology 15: 54.

(8) GARBER, R. J.
1922. INHERITANCE AND YIELD WITH PARTICULAR REFERENCE TO RUST RESISTANCE AND PANICLE TYPE IN OATS. Minn. Agr. Expt. Sta. Tech. Bul. 7, 62 p., illus.

(9) HARRINGTON, J. B., and AAMODT, O. S.
1923. THE MODE OF INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS WITH RELATION TO SEED COLOR IN CROSSES BETWEEN VARIETIES OF DURUM WHEAT. Jour. Agr. Research 24: 979–996, illus.

(10) HAYES, H. K., PARKER, J. H., and KURTZWEIL, C.
1920. GENETICS OF RUST RESISTANCE IN CROSSES OF VARIETIES OF TRITICUM VULGARE WITH VARIETIES OF T. DURUM AND T. DICOCCUM. Jour. Agr. Research 19: 523–542, illus.

(11) ——— and AAMODT, O. S.
1923. A STUDY OF RUST RESISTANCE IN A CROSS BETWEEN MARQUIS AND KOTA WHEATS. Jour. Agr. Research 24: 997–1012, illus.

(12) MAINS, E. B., and LEIGHTY, C. E.
1923. RESISTANCE IN RYE TO LEAF RUST, PUCCINIA DISPERSA ERIKSS. Jour. Agr. Research 25: 243–252, illus.

(13) ———
1924. NOTES ON GREENHOUSE CULTURE METHODS USED IN RUST INVESTIGATIONS. Ind. Acad. Sci. Proc. (1923) 33: 241–257, illus.

(14) MELCHERS, L. E., and PARKER, J. H.
1922. INHERITANCE OF RESISTANCE TO BLACK STEM RUST IN CROSSES BETWEEN VARIETIES OF COMMON WHEAT (TRITICUM VULGARE). (Abstract) Phytopathology 12: 31–32.

(15) ——— and PARKER, J. H.
1922. RUST RESISTANCE IN WINTER-WHEAT VARIETIES. U. S. Dept. Agr. Bul. 1046, 32 p., illus.

(16) NILSSON-EHLE, H.
1911. KREUZUNGSUNTERSUCHUNGEN AN HAFER UND WEIZEN. II. Lunds Univ. Årsskr., n. F., Afd. 2, Bd. 7, no. 6, 82 p.

(17) PARKER, J. H.
1920. A PRELIMINARY STUDY OF THE INHERITANCE OF RUST RESISTANCE IN OATS. Jour. Amer. Soc. Agron. 12: 23–38, illus.

(18) PUTTICK, G. F.
1921. THE REACTION OF THE F_2 GENERATION OF A CROSS BETWEEN A COMMON AND A DURUM WHEAT TO TWO BIOLOGIC FORMS OF PUCCINIA GRAMINIS. Phytopathology 11: 205–213.

(19) STAKMAN, E. C., and LEVINE, M. N.
1922. THE DETERMINATION OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON TRITICUM SPP. Minn. Agr. Expt. Sta. Tech. Bul. 8, 10 p., illus.

(20) VAVILOV, N. I. (WAWILOW, N.)
1913. BEITRÄGE ZUR FRAGE ÜBER DIE VERSCHIEDENE WIDERSTANDSFÄHIGKEIT DER GETREIDE GEGEN PARASITISCHE PILZE. Trudy Selek. Stan. Moskov. Selskokhoz Inst. 1: 1–108, illus. [In Russian. German résumé, p. 90–108. Translation in U. S. Dept. Agr., Bur. Plant. Indus.]

(21) WALDRON, L. R.
1921. INHERITANCE OF RUST RESISTANCE IN A FAMILY DERIVED FROM A CROSS BETWEEN DURUM AND COMMON WHEAT. N. Dak. Agr. Expt. Sta. Bul. 147, 24 p., illus.

INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI (BJERK.) WINTER, IN WHEAT[1]

By FRED N. BRIGGS[2]

Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The inheritance of resistance to disease in plants has been investigated but little as compared with the inheritance of morphological characters. Resistance to disease has been observed to segregate in simple Mendelian ratios in some instances, but in many cases the results have indicated that a comparatively large number of factors are involved, though the exact number has not been determined.

This paper deals with the genetics of resistance to bunt, *Tilletia tritici*, in two varieties of common wheat (*Triticum vulgare*), Martin and Hussar. These varieties were found to be free from bunt infection over a period of two years in an investigation of the relative resistance of wheat varieties to bunt conducted cooperatively by the United States Department of Agriculture and the agricultural experiment stations of the Pacific Coast States (*19*).[3] Further investigations indicate that these two varieties may reasonably be considered completely resistant, since the disease has not developed in them under conditions favorable for infection, during a period of five years. Most cultivated wheats are very susceptible to bunt, and a few varieties are highly resistant, but these two varieties are the only ones that have been completely resistant.

REVIEW OF THE LITERATURE

Differences in resistance of varieties of wheat to bunt were reported by Farrer (*8*) in 1901. Varietal resistance of wheat to bunt has been the subject of a number of investigations since that time, but these papers will not be reviewed here, as Reed (*17*) recently gave a rather complete review. In addition to Reed's paper, a very comprehensive investigation (*19*) of the relative resistance of wheat varieties to bunt was published in 1925. In the fall of 1918 the United States Department of Agriculture, in cooperation with the California, Oregon, and Washington agricultural experiment stations, undertook an investigation of the relative resistance of varieties of wheat to bunt. The trials included practically all of the commercial wheats of the United States, as well as numerous other varieties and pure-line selections from the wheat classification nurseries of the Office of Cereal Investigations of the United States Department of Agriculture.

[1] Received for publication Aug. 19, 1925; issued May, 1926. These investigations were conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the California Agricultural Experiment Station.

[2] Throughout this investigation the writer received many suggestions from R. E. Clausen and J. L. Collins, Division of Genetics, and W. W. Mackie, Division of Agronomy, University of California, and from various members of the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and he wishes here to acknowledge their valuable assistance.

[3] Reference is made by number (italic) to "Literature cited," p. 990.

W. W. Mackie and the writer conducted the investigations in California, where a total of 956 varieties and strains were grown. In 1920, when the most severe infection was obtained, the disease appeared on more than 75 per cent of the plants in 811 varieties and strains. At the same time only 42 varieties and strains had less than 25 per cent of the plants attacked by bunt.

Farrer (*8*), in 1901, seems to have been the first to attempt to bring about bunt resistance in wheats by hybridization. Having observed that 10 Australian varieties carried infections varying from 12 to 95.5 per cent, he thought that a similar degree of variation might occur in the hybrid generations of his crosses. His plan was to subject F_2 and F_3 to a heavy bunt attack and pick out the bunt-free plants. He made no attempt to observe the segregation into resistant and susceptible forms in order to establish Mendelian ratios. Later (*9*) he reported that the best of his hybrid selections exhibited from 5 to 10 per cent of infection under the same conditions where such susceptible varieties as Tuscan, Purple Straw, Steinwedel, White Lammas, White Essex, Golden Drop, and others showed from 80 to 100 per cent. In 1905 (*10*) Farrer stated that he made crosses the previous season for the special purpose of getting bunt-resistant varieties. Evidently he then used for the first time parents which were known to be bunt resistant. Sutton, who had been Farrer's assistant, continued the breeding of wheats resistant to bunt after Farrer's death in 1906. Two varieties, Florence and Genoa, which are highly resistant, were produced. Sutton (*18*) states that these two are full sisters, and came from a cross made in 1901.

Pye (*16, p. 373*) made numerous crosses with Medeah, a durum, which he found to be highly resistant to bunt. He had no difficulty in producing varieties that were highly resistant, but he did find it difficult to produce one that, in addition to having resistance, met the practical and economic needs of the farmer and the miller. Pye's difficulty was due, no doubt, to the fact that his crosses were between species of wheat, *Triticum durum* and *T. vulgare*.

Except for the work in Australia, it seems that no attempts were made to breed wheats resistant to bunt, or to study the genetics of the problem, until Gaines (*11*) began his investigations in 1915. It seems rather strange that a genetical study was not undertaken before this time, because a large number of investigators had observed great differences in susceptibility, and Biffen (*4, p. 40–44*) had shown as early as 1905 that resistance to a plant disease may be inherited in a simple Mendelian manner.

Gaines (*12*) studied the genetics of resistance in the following types of crosses: (1) Resistant × resistant, (2) resistant × susceptible, and (3) susceptible × susceptible. The most susceptible wheats, sown under conditions favoring maximum infection, had an average of about 80 per cent of bunted heads. The 20 per cent of sound heads seemed to be due to accident, for in crosses with other varieties the descendants did not show evidence of having inherited any cumulative resistance from the susceptible parent. Gaines placed Hybrid 128 and Jones Winter Fife in the susceptible group. Fortyfold, Red Russian, and Marquis each had differing dilute resistances, which reduced the amount of bunt by 10 to 25 per cent. When added together as in crosses between them, the

cumulative effect made a more concentrated resistance, having a value of from 30 to 60 per cent. Marquis was much more resistant when sown in the spring. Turkey, Florence, and Alaska each had differing concentrated resistances, which reduced the amount of bunt 70 to 75 per cent, compared with the standard susceptible varieties. These concentrated resistances also were cumulative in effect when brought together by crossing, the resulting descendants segregating into immune, very resistant, various stages of dilute resistant, and completely susceptible classes. Although Gaines demonstrated that different factors for resistance were present, he was unable to determine definitely the number of factors or to determine with certainty the effect of any one factor acting alone.

In summing up the work of previous investigators, it will be seen that breeding for bunt resistance, without reference to its genetics, has resulted in the production of commercial varieties of wheat which are resistant to bunt. Gaines seems to have made the only attempt to investigate the genetics of resistance to bunt.

METHODS

The parental material and the hybrid progenies were grown in the field at the University Farm, Davis, Calif. Conditions there were especially suitable for these investigations because relatively high bunt infections can be obtained when wheat is sown in the fall. Both spring and winter varieties may be seeded at that time without any danger of winterkilling, and with the assurance that both types will mature during the following summer. Hybrids between spring and winter wheats, therefore, may be grown without any danger of having a part of the population winterkilled. As most of the crosses reported in this paper were between spring and winter varieties, this was of considerable importance.

The seeds were thoroughly inoculated with *Tilletia tritici* by placing an excess quantity of the spores in a glass container with the seed wheat and shaking vigorously. The inoculum was originally collected from a rod row of Little Club wheat and grown each succeeding year on White Federation wheat. The inoculum, therefore, was all grown from one original collection of bunt. This was not done because it was suspected that physiological races of bunt existed, but because this method offered an easy and a definite source of inoculum. Faris (7) recently reported preliminary results which indicate that physiological races of bunt exist. To what extent these occur is not known. The fact that the same collection of bunt was used in all these present experiments makes it quite certain that the same race or the same mixture of races was employed. This is indicated also by the constant way in which the parental material behaved toward this collection of bunt.

The seed was sown by hand in rod rows 12 inches apart, the seeds being spaced from 2 to 3 inches apart to facilitate the separation of plants at harvest. The entire nursery was sown within 3 or 4 days in order to avoid the effects of changing temperature and moisture conditions.

The nursery always was sown in a field which had been summer-fallowed the previous season, so that it was almost entirely free from volunteer grain. At harvest time, the plants in each row were

pulled and were separated into two piles: Bunt-free and bunted. The total number of plants and the number of bunted plants were recorded and the percentage of bunt calculated. A plant was classed as bunted if it showed any infection whatever. Gaines (*12, p. 460*) separated the plants into three piles: Bunt-free, all bunted, and partly bunted. The computation method which he used gave the total bunt in terms of percentage of the total plants in the row, according to the formula $ab+c=d$. In this formula, a is the percentage of bunted heads on the partly bunted plants, b is the percentage of partly bunted plants in the row, c is the percentage of entirely bunted plants in the row, and d is the total percentage of bunted plants in the row. His method gives a satisfactory quantitative measure of resistance, but does not indicate the nature of segregation within a given row because the plant is not maintained as the unit. It seems that the plant should be considered as the unit in a study of inheritance.

PARENT STOCK

Five varieties of common wheat (*Triticum vulgare*) were used in this investigation. Three varieties—Hard Federation, White Federation, and Baart—are highly susceptible to bunt. The other two varieties—Martin and Hussar—are completely resistant. The resistant varieties are winter wheats, and the susceptible ones are spring wheats. A full description and history of these five varieties has been given by Clark, Martin, and Ball (*6*). The bunt infection in the parent varieties over a period of five years is recorded in Table I.

TABLE I.—*Annual percentage of bunt infection in the parent wheat varieties from 1920 to 1924*

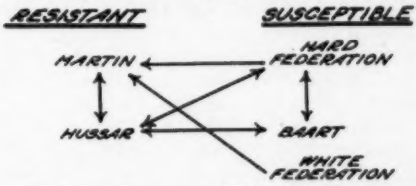
Variety	Percentage of bunted plants					
	1920	1921	1922	1923	1924	Average
Martin	0.0	0.0	0.0	0.0	0.0	0.0
Hussar	0.0	0.0	0.0	0.0	0.0	0.0
Hard Federation	89.7	62.4	53.3	60.4	82.1	69.6
White Federation	88.7	51.6	58.3	75.3	84.9	71.8
Baart	95.7	68.2	81.7	84.2	88.2	83.6

There is considerable variation in the percentage of bunt in the susceptible varieties from year to year. This fluctuation is due to seasonal differences at the time of planting, and is by no means unexpected. In general, the relative position of the varieties was maintained, regardless of the actual percentage of infection. Baart consistently showed a higher percentage of infection than either Hard Federation or White Federation. During the last three years, White Federation was more susceptible than Hard Federation. In 1921, Hard Federation was more susceptible than White Federation, while in 1920 the infection was practically the same. During the first four years the percentage of infection is based on average results from 4 to 6 rod rows, whereas in 1924 it is based on about 40 rows in the case of Hard Federation and Baart and 16 rows in the case of White Federation. The data for 1924, therefore, are considered as being more reliable. The two resistant varieties were entirely free from bunt throughout the five-year period.

EXPERIMENTAL RESULTS

The following diagram shows how the crosses with the five wheats were made:

Reciprocal crosses were made where the arrow points in both directions; in the other cases the cross was in one direction only. Except that White Federation replaces Baart in the cross with Martin, and no reciprocal cross was made between Martin and Hard Federation, all the possible crosses were made between Martin, Hussar, Hard Federation, and Baart. Reciprocal crosses did not behave differently, and for the most part they will be treated together.



The crosses were subjected to bunt infection in F_1,[3] F_2, and F_3.

BUNT INFECTION IN F_1

Because of the tedious process of making hybrids in wheat, the investigation of F_1 was extended over a period of three years, beginning in 1922. The results are shown in Table II.

TABLE II.—*Percentage of bunted plants in the F_1*

Year	Hybrid No.	Cross or parent	Number of plants		Percentage of bunt infection [a]	
			Total	Bunted	Actual	Average
1922	.....	Baart	121	101	83.47	.....
	.....	Hard Federation	86	49	56.97	.....
	.....	Hussar	113	0	0.0	.....
	21228	Baart×Hard Federation	157	103	65.60	66.26
	21229	Hard Federation×Baart	130	87	66.92	
	21230	Hard Federation×Hussar	32	0	0.0	0.47
	21231	Hussar×Hard Federation	105	1	0.95	
	21232	Baart×Hussar	131	1	0.76	0.77
	21233	Hussar×Baart	128	1	0.78	
1923	.....	White Federation	76	58	76.31	.....
	.....	Martin	91	0	0.00	.....
	.....	Hussar	72	0	0.00	.....
	21240	White Federation×Martin	123	0	0.00	0.00
	21241	Martin×White Federation	106	0	0.00	
	21236	Hussar×Martin	128	0	0.00	0.00
	21237	Martin×Hussar	118	0	0.00	
1924	.....	Hard Federation	92	75	81.52	.....
	.....	Martin	141	0	0.00	.....
	23042	Hard Federation×Martin	42	0	0.00	0.00
	23041	Martin×Hard Federation	41	0	0.00	

[a] The percentage of infection in the parents in this table is calculated from data on rod rows grown adjacent to the rows of F_1 plants.

F_1 of the cross between susceptible varieties, Baart×Hard Federation, was somewhat intermediate between the two parents. From the behavior in F_3, this does not seem to be of any significance. F_1 of the cross between Hard Federation and Hussar was almost free from bunted plants. One plant out of 137 was infected. Also

[3] The complete study of F_1 was made possible through the courtesy of V. H. Florell, associate agronomist of the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, who furnished some of the hybrid seed.

one plant in each of the two rows of the cross between Baart and Hussar showed infection. Resistance to bunt infection may be considered, therefore, as almost completely dominant. The fact that 3 plants out of 396 became infected indicates that plants which inherit resistance from a parent like Hussar occasionally may become infected. F_1 of both Martin × Hard Federation and Martin × White Federation crosses were free from bunt infection, indicating that the resistance of Martin to bunt is completely dominant. If the Martin F_1 had been planted the previous year under the same conditions as the Hussar F_1, a few plants might have been infected. On the other hand, this slight difference may be due to the presence of modifying factors. The difference in any case is very slight, and for the purposes of this investigation both will be considered as dominant. Finally, F_1 of Hussar × Martin was free from bunt infection, as would be expected from the behavior of the other crosses.

SEGREGATION IN F_2

All F_2 progenies were grown in 1923. It was realized in the beginning that F_2 would not give data from which the mode of Mendelian inheritance of resistance could be worked out, because 100 per cent of the plants in a rod row rarely ever are infected in the most susceptible varieties. If bunt-free plants from a susceptible variety like Baart are saved and the seed sown the next season, they will be found not to differ in their resistance from the unselected parent variety, showing that the bunt-free plants escaped infection and do not differ in susceptibility from those that exhibit the disease. F_2 data are valuable because they give some indication as to the number of factors present and as to the behavior of the different genotypes. Data on F_2 progenies in 1923 are recorded in Table III. The percentage of bunt in the parents in 1923 is taken from Table I.

TABLE III.—*Percentage of bunted plants in F_2*

Hybrid No.	Parents and cross	Number of plants		Percentage of bunted plants
		Total	Bunted	
	Martin			0.0
	Hussar			0.0
	Hard Federation			60.4
	White Federation			75.3
	Baart			84.2
21228	Baart×Hard Federation	2387	1854	77.7
23041	Martin×Hard Federation	830	159	19.2
21241	Martin×White Federation	609	105	17.2
21231	Hussar×Hard Federation	2159	210	9.7
21233	Hussar×Baart	1877	183	9.7
21236	Hussar×Martin	1543	0	0.0

F_2 of Baart × Hard Federation was infected to a degree intermediate between that of the two parents. This fact appears to have no significance, as will be brought out in connection with the results obtained in F_3.

F_2 of Martin × Hard Federation produced 19.2 per cent of bunted plants, and Martin × White Federation 17.2 per cent. Knowing that some plants in a susceptible variety normally escape infection,

and assuming that the same thing happens in hybrid plants, the above percentages suggest that the resistant parent differs from the susceptible parent in one dominant factor. That is, it may be assumed that enough susceptible plants escaped infection to bring the total susceptible plants up to 25 per cent. Data obtained in F_3 confirm this assumption.

F_2 of both the Hussar crosses produced 9.7 per cent of bunted plants, which is considerably less than the percentage obtained in the Martin crosses. These data indicate, then, that Hussar differs from the susceptible parents in more than one factor. As it is not possible to obtain the exact percentage of susceptible progeny from F_2 data, neither the number of factors nor the exact effect of each one can be determined. The amount by which 9.7 per cent will be increased by escaped susceptible plants may be compensated for, in part at least, by the fact that a few resistant segregants may become infected as indicated by F_1 data. As F_2 of Hussar × Martin did not produce any susceptible progeny, it is assumed that they have one factor in common for resistance. This point was investigated further in F_3.

SEGREGATION IN F_2

All F_3 progenies except Hussar × Hard Federation were grown in 1924. Rather large F_3 populations were grown because the classification of F_2 plants, based on the behavior of their progenies in F_3 rows, is more reliable than that based on the F_2 plants themselves. A given susceptible F_2 plant may escape infection, but a whole row of plants from seed of such an F_2 would hardly escape infection in F_3. Seeds from a single F_2 plant were sown for each F_3 rod row. Approximately one-half million F_3 plants, in more than 5,500 rod rows, were grown, classified, and counted.

Baart × Hard Federation

Two hundred F_3 rows were grown from seed representing 200 F_2 plants which had been protected from bunt infection in F_1 and F_2 by disinfecting the seed with copper carbonate. In this series, then, all of the progeny were present. Another series of 200 rod rows was grown from seed of 200 plants which were not infected in F_2. It was shown in Table III that 22.3 per cent of the F_2 plants from this cross did not exhibit the disease. It was thought that if these two varieties differed in any factors for resistance, the uninfected F_2 plants should show a certain amount of increased resistance due to the recombination of these factors. In that case the latter series should show a greater proportion of rows with a low percentage of bunt than the series from F_2 plants which were protected from bunt. Forty-four rod rows of Hard Federation and also 40 rows of Baart were grown in order to get a more accurate measure of parent variability, with results as recorded in Table IV.

The difference in mean bunt infection between Hard Federation and Baart is 6.10 ± 0.81, which is more than seven times the probable error. There is no evidence, however, of greater resistance in the hybrid series which was subjected to bunt infection in F_2. Neither is there any evidence of forms more resistant or more susceptible than the parents. The coefficient of variability is about the same for hybrid rows as for parent rows. The differences in infection

between Hard Federation and Baart no doubt is due to modifying factors which are so universally present, but as they exert only a relatively small effect they may be disregarded in working out the main factor differences between susceptible and resistant varieties. Hard Federation and Baart, then, may be considered completely susceptible, as contrasted with resistant Martin and Hussar.

TABLE IV.—*Distribution of F_3 rows into 5 per cent classes for bunt infection*

Parent or cross	Distribution of rows by percentage classes of bunt infection in F_3										Number of rows	Average percentage of bunt		
	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100		Mean	Standard deviation	Coefficient of variability
Hard Federation	----	----	1	1	4	11	11	10	6	----	44	82.1	6.62	8.06
Baart	----	----	----	----	----	3	7	17	11	2	40	88.2	5.00	5.67
Baart × Hard Federation:														
F_2 not subjected to infection	----	----	2	4	7	10	31	60	67	19	200	87.9	6.84	7.78
F_2 subjected to infection	----	----	----	----	1	10	27	57	67	38	200	89.8	5.50	6.12

MARTIN × HARD FEDERATION, AND MARTIN × WHITE FEDERATION

Two series of each of the crosses with Martin were grown in F_3. One series was grown from seed from an F_2 population where the plants were protected from bunt infection by seed treatment, so all of the progeny were present. The other series was from plants subjected to infection in F_2, so only those plants which survived the bunt attack were represented. This method was followed for two reasons: (1) To see whether any susceptible plants had escaped infection in F_2; (2) to try to fix the lower limit of the susceptible class as it appeared in F_3 rows. The data covering these crosses are presented in Table V. The rows within the 0-to-5 per cent class were separated into two parts: (1) Those rows which had no bunt; (2) those which had from 1 to 5 per cent. This was done because the former rows are of special interest.

The nature of the distribution may be seen better in Figures 1 and 2. Because there were considerable differences in their sizes, the populations were reduced to a comparable basis by dividing each class by the total number of rows and multiplying by 100. It may be considered, therefore, that each curve represents the distribution of 100 rows, based on the actual distribution of the number of rows shown in Table V.

The solid lines in Figures 1 and 2, which represent the distribution of populations where all types of F_2 progeny were present, form distinctly trimodal curves. The distribution of rows under the three modes agrees very closely with the 1:2:1 ratio. In both cases the number of rows between 0 and the first minimum point of the curve is almost exactly one-fourth of the total. The number between the first and second minima amounts to about two-fourths of the total. The second minimum is taken as 50 per cent, because in considering both curves it represents roughly the lowest point. Also, if the broken-line curves are considered, 50 per cent roughly marks

the point where the curve in the susceptible region begins to fall off, because of the fact that a large number of the susceptible plants were eliminated in the previous generation. The last section of the curve includes the remaining one-fourth of the population. The deviation in the case of Martin × Hard Federation is 3.10 ± 2.92, which is less than two times its probable error, while in the case of Martin × White Federation the deviation is 1.40 ± 2.92, which is less than its probable error. If 40 or 60 per cent instead of 50 per cent had been taken as the point for the second minimum, the deviation still would have been well within three times the probable error.

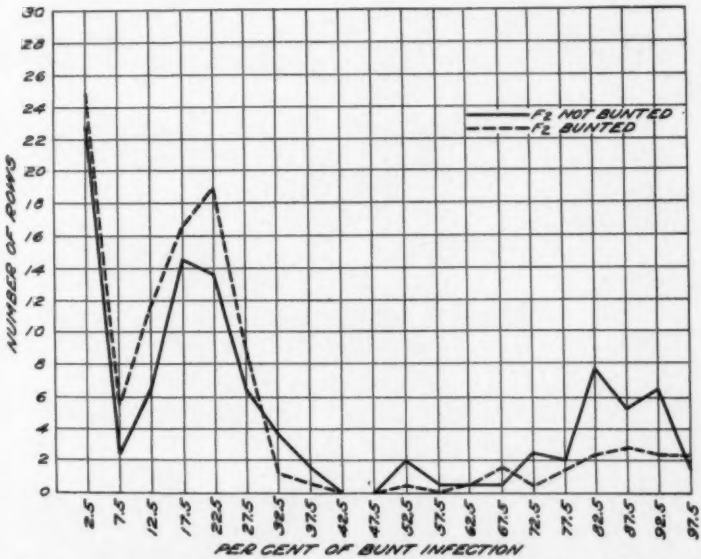


FIG. 1.—Per cent of bunt infection, Martin×Hard Federation cross. Distribution of F_2 plants on basis of F_3 rod rows. Solid line: F_3 not bunted. Broken line: F_3 bunted

TABLE V.—*Distribution of F_3 rows into 5 per cent classes for bunt infection*

Parent or cross	Distribution of rows by percentage classes of bunt infection in F_3																					Total number of rows
	0	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100	
Hard Federation														1	1	4	11	11	10	6		44
White Federation																	3	6	3	4		16
Martin×Hard Federation:																						
F_2 not subjected to infection	41	15	6	16	36	34	16	9	4			5	1	1	1	6	5	19	13	16	4	248
F_2 subjected to infection	66	8	17	35	50	56	20	4	1			1		1	5	1	4	7	8	4	4	298
Martin×White Federation:																						
F_2 not subjected to infection	51	20	4	16	43	43	27	10	3	2	1	2	3	2	3	6	6	19	15	13	10	290
F_2 subjected to infection	67	13	24	41	57	48	16	6	1		2		1			1	4		2	4	1	288

A study of the broken-line curves, which represent the populations where F_2 was subjected to bunt infection and the infected plants eliminated, shows that some of the susceptible plants were not infected in F_2. The question naturally arises as to whether or not there are enough susceptible plants which escaped infection in F_2 to bring the susceptible class of F_2 up to the expected 25 per cent. In the case of Martin × Hard Federation F_2, infection was 19.2 per cent, based on a total population of 830 plants (Table III). However, only seed of the first 298 plants that survived the bunt attack was sown. That portion of F_2 which produced these 298 bunt-free plants also included 55 bunted plants. As shown in Table V, 35 susceptible plants escaped F_2 infection, thus giving in reality a total of 90 susceptible plants in an F_2 population of 353. When corrected on the basis of F_3 results there were 25.5 per cent of susceptible plants

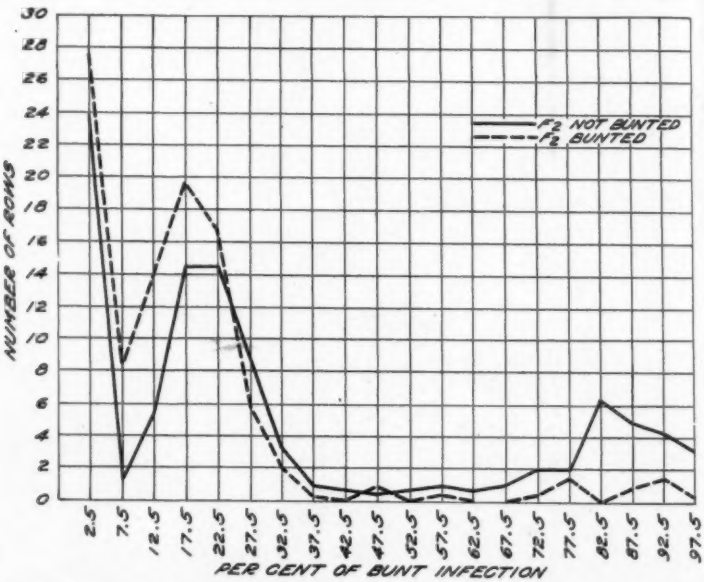


FIG. 2.—Per cent of bunt infection, Martin × White Federation cross. Distribution of F_3 plants on basis of F_2 rod rows. Solid line: F_2 not bunted. Broken line: F_2 bunted

in F_2, which is very near the 25 per cent expected on the basis of one dominant-factor difference. Likewise, in Martin × White Federation there were 62 bunted plants in that part of the F_2 population which produced the first 288 bunt-free plants, and, as indicated in Table V, 13 susceptible plants which escaped F_2 infection, making a total of 75 susceptible plants in an F_2 population of 350. As corrected, 21.4 per cent of the plants in F_2 were susceptible. The deviation is 12.50 $\pm$5.46 plants, which is 2.3 times its probable error. Segregation of F_2 plants on the basis of the behavior of their progeny in F_3 rows, and the corrected percentage of bunt in F_2, both indicate that Martin differs from Hard Federation and from White Federation in one main dominant factor for resistance to bunt.

Hussar×Hard Federation, and Hussar×Baart

A very large F_3 population of Hussar×Hard Federation was grown in 1923. V. H. Florell had made this cross for another study before this problem was undertaken, and his courtesy in furnishing F_2 plants made it possible to investigate the F_3 of this cross one year in advance of other F_3 populations. Because the Hussar crosses presented a more complex situation than the Martin crosses, as indicated by F_2 results, larger populations were grown. Except for the one population mentioned above, all F_3 populations were grown in 1924. As in the crosses with Martin, two series were grown. One series had been protected from previous infection so that it represented a random sample of all types of progeny. The other series was subjected to bunt infection in F_2, with the result that only those plants which survived the attack were present. Data from the F_3 of these crosses are presented in Table VI.

Table VI.—*Distribution of F_2 rows into 5 per cent classes for bunt infection*

Parent or cross	Distribution of rows by percentage classes of bunt infection in F_3																				Total number of rows
	0	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100	
Hard Federation														1	1	4	11	11	10	6		44
Baart																	3	7	17	11	2	40
Hussar×Hard Federation:																						
F_2 not subjected to infection	810	263	302	267	149	91	70	50	49	37	32	36	33	22	13	14	10	3	5	2		2,258
F_2 subjected to infection	259	86	88	59	37	28	15	10	7					1	1	1		2			2	596
Hussar×Baart:																						
F_2 not subjected to infection	214	99	73	56	40	37	15	8	10	10	4	3	2	5	4	1	4	7	1	6	1	598
F_2 subjected to infection	270	89	88	56	32	15	9	3	1	2	1	2	1	2				4	3	2		580

The segregation of F_3 rows as shown in Table VI is quite different from that of the Martin crosses shown in Table V. Here again, because of the great difference in the sizes of populations, the results are represented graphically in Figures 3 and 4 on the basis of 100 rows. The solid lines in Figures 3 and 4 represent the distribution of F_2 plants based on the behavior of their F_3 progeny where all types of the progeny were present. The broken lines represent the distribution where F_2 was subjected to bunt attack and the bunted plants eliminated. A few susceptible plants escaped infection in F_2.

It will be recalled that 9.7 per cent of bunt occurred in F_2. In Hussar×Hard Federation, that part of the population which produced the 596 bunt-free plants included 61 bunted plants. These, with the 7 susceptible plants which escaped infection in F_2, as shown by Table VI, make a total of 68 susceptible plants in a population of 655. As corrected, then, there were 10.4 per cent of susceptible plants in F_2. Correcting the classification of F_2 of Hussar×Baart in the same way, there were 11.7 per cent of susceptible plants. Even when it is considered that about 0.8 per cent of F_1 plants became infected, the above figures are too high to be accounted for by duplicate factors, which would theoretically give 6.25 per cent of bunt. For Hussar×Hard Federation the deviation is 21.60 ± 4.18, which is 5.17 times its probable error; and for Hussar×Baart it is 35.00 ± 4.13,

which is 8.5 times its probable error. What then must be the constitution of Hussar in order to produce 10 or 11 per cent of infection in F_2?

As no susceptible segregates appeared in F_2 of Martin × Hussar, evidently the factor for resistance in Martin also is present in Hussar. The F_3 data, which will be presented later, confirm this conclusion. If it be assumed that a second factor in the heterozygous condition permits an infection intermediate (about 40 per cent) between that of the resistant and the susceptible parent, the agreement in F_2 is very close. For Hussar × Hard Federation the deviation is 5.80 ± 4.18, which is 1.4 times its probable error; and for Hussar × Baart it

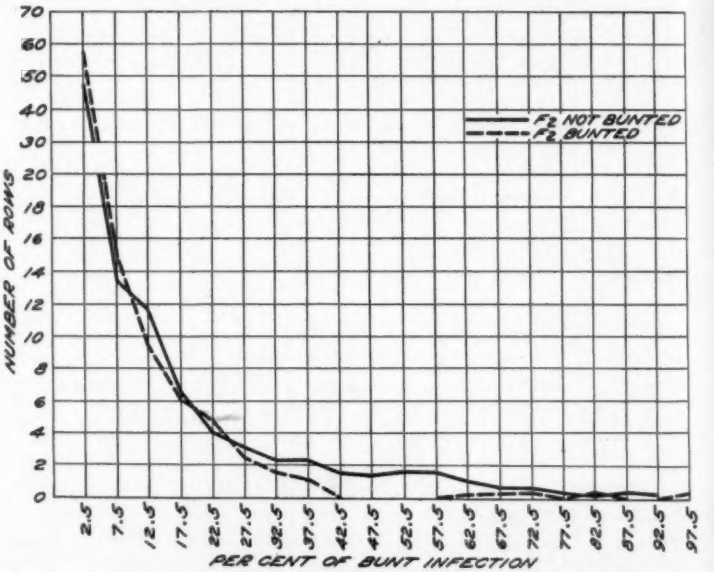


FIG. 3.—Per cent of bunt infection, Hussar × Hard Federation cross. Distribution of F_2 plants on basis of F_3 rod rows. Solid line: F_2 not bunted. Broken line: F_2 bunted.

is 3.00 ± 4.13, which is less than its probable error. Will multiple factors of the above sort give the segregation of F_2 plants on the basis of F_3 rows as recorded in Table VI? If the first factor be designated by R, which is completely dominant as the factor present in Martin, and the second by S, which gives an intermediate condition when heterozygous, the genotypic constitution of F_2 plants and the percentage of bunt expected in F_3 rows is as follows:

F_2 genotypes	Behavior in F_3 rows
1. RRSS	Completely resistant.
2. RRSs	Do.
2. RrSS	Do.
4. RrSs	Same as Hussar F_2, about 9 or 10 per cent of bunt.
1. RRss	Completely resistant.
2. Rrss	Same as Martin F_2, about 18 or 19 per cent of bunt.
1. rrSS	Completely resistant.
2. rrSs	Intermediate between resistant and susceptible parents, or about 40 per cent of bunt.
1. rrss	Completely susceptible, or about 80 per cent of bunt.

The phenotypic ratio is 7 completely resistant : 4 like Hussar F_2 (9–10 per cent) : 2 like Martin F_2 (18–19 per cent) : 2 intermediate between two parents (40–45 per cent) : 1 completely susceptible. It is difficult to determine to what extent all of the above classes have been realized, because the limits of the intermediate classes are not definitely marked. It is possible to determine to what extent completely resistant and completely susceptible classes have been obtained. The resistant class of Hussar × Hard Federation where the F_2 was not bunted (Table VI) should contain 987 rows. In order to complete this class, 177 of the highly resistant rows have to be taken from the 1 to 5 per cent group. This was anticipated, because in the Martin crosses it was necessary to take all of the rows having up to 10 per cent of infection to fill out the resistant class.

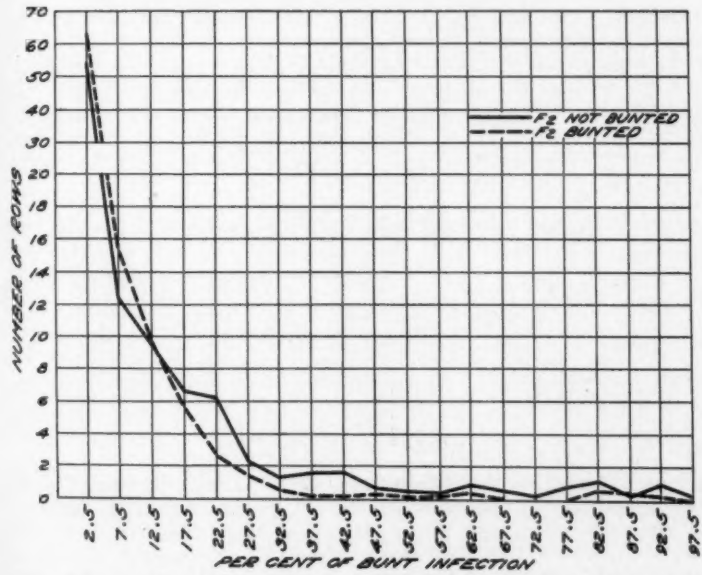


FIG. 4.—Per cent of bunt infection, Hussar × Early Baart cross. Distribution of F_2 plants on basis of F_3 rod rows. Solid line: F_2 not bunted. Broken line: F_2 bunted.

That genotypically resistant plants may exhibit slight infection is indicated by the behavior of about 700 F_4 rows of Hussar × Hard Federation. These were grown from plants selected for agronomic purposes and came from F_3 rows which were entirely free from bunt. A number of these F_4 rows showed a slight infection, estimated in most cases to be less than 10 per cent, but counts were not made to determine the exact percentage. It is entirely possible that modifying factors may be responsible for the fact that some of the plants which are expected to be free from bunt actually show a slight infection. Investigations now under way should clear up this point. In order to complete the resistant class of Hussar × Baart, 45 of the 99 rows in the 1 to 5 per cent group had to be used. In general, then, the resistant class agrees fairly well with the expectation in each case.

The other class that can be determined with a fair degree of certainty is the completely susceptible one. If all rows that produced 50 per cent or more of bunt are considered susceptible, as was done in the case of Martin, the expected one-sixteenth is obtained within very close limits. In the case of Hussar × Hard Federation there were 138 where 141 were expected, a deviation of 3.00 ± 7.75, which is less than its probable error. In Hussar × Baart 34 were obtained where 37 were expected, a deviation of 3.00 ± 3.99, which is less than its probable error. The observed number of completely susceptible rows therefore was a very close approximation to the 1:15 ratio.

It is more difficult to get at the class limits for the other three phenotypes. If it be assumed that the RrSs individuals behave as F_2 of the Hussar crosses, the Rrss individuals behave as F_2 of the Martin crosses, and the rrSs are intermediate between the two parents with the mode at about 40 per cent of infection, a fair idea of the sort of curve expected in that region may be constructed in part on the basis of F_2 rows. In each of the Hussar crosses there were 30 rod rows containing the entire F_2 population, and in each of the Martin crosses 15 rod rows. That there was considerable variation from row to row in the percentage of bunt will be seen in Table VII.

TABLE VII.—*Distribution of F_2 rows into 5 per cent classes for bunt infection*

Cross	Distribution of rows by percentage classes of bunt infection in F_2								Number of rows
	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	
Martin × Hard Federation	------	3	2	4	3	1	1	1	15
Martin × White Federation	------	3	3	4	2	2	1	------	15
Hussar × Hard Federation	3	13	11	2	1	------	------	------	30
Hussar × Baart	4	11	10	3	2	------	------	------	30

If 60 F_2 rows of the Hussar crosses are taken to represent the four-sixteenths of the population from RrSs plants and 30 F_2 rows of the Martin crosses to represent the two-sixteenths of the population from Rrss plants, a curve may be constructed which should be the type of curve expected in this region. There would be two-sixteenths of the population from rrSs plants which should have a mode near 40 per cent. This part of the curve is entirely speculative. A curve constructed on this basis is given in the upper chart of Figure 5. The region from 7.5 to 27.5 per cent agrees very well with the actual curves in Figures 3 and 4. The region from 27.5 to 52.5 does not agree with the actual ones. An alternative explanation, based on the segregation of F_3 rows, without special emphasis on the percentage of bunt in F_2, would be duplicate genes, either one of which acting alone would give results similar to those obtained with Martin. In that case four-sixteenths of the population would be like F_2 of the Hussar crosses, and four-sixteenths would be like F_2 of the Martin crosses. A curve constructed on this basis is given in the lower chart in Figure 5. Here the agreement with the actual distributions shown in Figures 3 and 4 is much better. On this assumption, 6.25 per cent of bunt would be expected in the F_2 genera-

tion. A deviation of 3 times the probable error would allow a maximum of 8.25 per cent, which is considerably less than that actually obtained.

The two alternative explanations offered above are the simplest ones possible, and, as noted, neither exactly meets the requirements of the actual occurrence. For the present it is assumed that Hussar very probably differs from Hard Federation and from Baart in two main factors for resistance to bunt, because the preponderance of evidence is in that direction. However, when data are available on which the factorial composition can be determined with certainty,

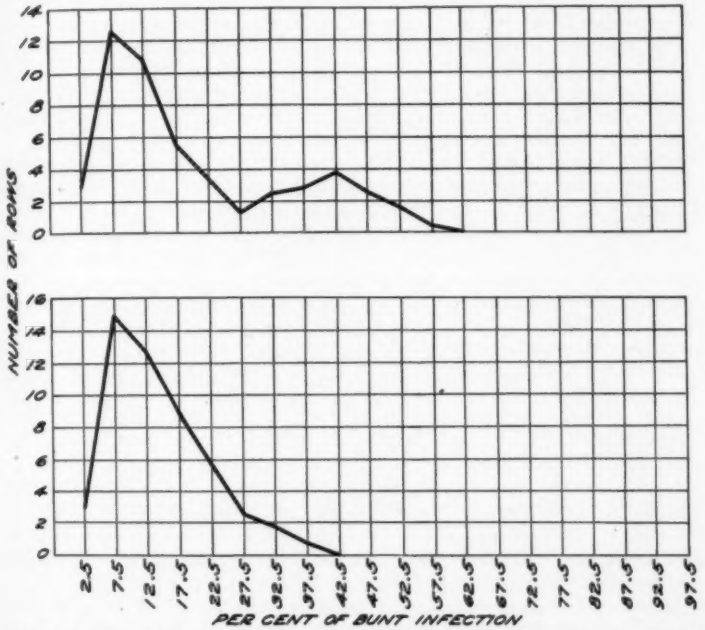


FIG. 5.—Per cent of bunt infection. Upper chart: Theoretical curve for the region between 5 and 50 per cent, where one factor is completely dominant and the other produces an intermediate condition when heterozygous. Lower chart: Theoretical curve for region between 5 and 50 per cent, where both factors are completely dominant.

it is entirely possible that the explanation may call for a larger number of factors. One factor is identical with that of Martin. The exact effect of the other factor, or perhaps factors, may be determined with certainty by continued investigations, because forms now may be isolated which lack the factor common to Martin and to Hussar.

MARTIN × HUSSAR

Four hundred F_2 plants were represented in F_3. It will be recalled that no bunt appeared in F_1 and F_2 of this cross. The F_3 population, likewise, contained none of the disease. This indicates that the factor for resistance to bunt in Martin is identical with one of the factors in Hussar. The constitution of Martin may be designated tentatively as RRss and that of Hussar as RRSS.

DISCUSSION

The inheritance of resistance to disease in plants has been explained in many instances on the basis of a relatively large number of factors. In most cases little effort has been made to isolate the various factors, with the result that practically nothing is known either as to the exact number or as to their specific effect. Biffen (*4, p. 40–44*) discovered in 1905 that resistance to yellow rust, *Puccinia glumarum*, in a cross between Rivet, a slightly susceptible, and Red King, a very susceptible variety, was recessive in F_1 but appeared in approximately one-fourth of F_2. The recessive forms bred true for resistance. Here the inheritance of resistance to disease segregated in a monohybrid ratio.

Perhaps the inheritance of disease resistance may reasonably be expected to be complicated, as it is the result of the interaction of two systems—the host plant, and the disease organism. When as much attention is paid to the purity of the fungus as to that of the host, the results may be considerably simplified. In fact, the genetics of stem-rust resistance presented just such a complicated situation until physiological races of the fungus were isolated and utilized. Aamodt (*1, 2*) inoculated in the greenhouse F_3 seedlings of a cross between Kanred and Marquis with one physiologic form of stem rust to which Kanred is immune and Marquis susceptible. Immunity was dominant and segregation was explained on the basis of a single-factor difference. Melchers and Parker (*15*) also obtained a monohybrid ratio in crosses between resistant Kanred and the susceptible varieties, Marquis, Preston, and Haynes Bluestem, where they were inoculated with a single strain of stem rust. Inoculations were made at the time of heading. Likewise, Burkholder (*5*) and McRostie (*13, 14*) studied the inheritance of resistance of beans to two strains, *alpha* and *beta*, of anthracnose, *Colletotrichum lindemuthianum*. Crosses between varieties resistant to the *alpha* strain and varieties susceptible to it segregated in F_2 into 3 resistant to 1 susceptible. Similar results were obtained with the *beta* strain. Crosses between a variety resistant to both strains with varieties susceptible to both strains gave the expected F_2 ratio of 9 resistant to 7 susceptible.

As already pointed out, Gaines (*12*) was able to explain the inheritance of resistance to bunt only on the basis of a number of factors. The influence of any single factor was not well defined. He accounts for his results with Turkey × Alaska on the basis of six factors, any one of which will reduce the amount of bunt by about 20 per cent. If Turkey resistance *abc* meets Alaska resistance *xyz* the progeny will produce from +65 to −55 per cent of the disease. Segregation in the cross between Turkey and Florence was similar to that in the cross between Turkey and Alaska.

As multiple factors, first discovered by Nilsson-Ehle, are met with so frequently, it was by no means a surprise to find them in connection with bunt resistance. Investigations of the inheritance of resistance to oat-smut infection indicate that multiple factors are present in oats also. Wakabayashi (*20*) studied the inheritance of resistance to covered smut, *Ustilago laevis*, in a cross between Red Rustproof and Black Tartarian oats. The former was completely resistant to this disease, while Black Tartarian had from 25 to 40 per cent of smut. The F_1 and F_2 did not contain any smut, but 12 F_3 rows out of a total of 107 produced from 0.1 to 15 per cent of the disease. He concluded

that the F_3 is nearly what is to be expected if the immunity in Red Rustproof is caused by three independent dominant factors, any one of which would prevent the appearance of covered smut. Evidently, Wakabayashi assumes that such F_2 genotypes as AaBbCc and AaBbcc do not produce any smut in F_3 rows, otherwise three factors will not explain his results. In a preliminary report of a study of the inheritance of resistance to loose smut (*Ustilago avenae*) in oats, Barney (*3*) suggests the presence of three independent dominant factors—S, S′, and S″—for resistance.

The genetics of disease resistance in plants may often appear more complex than it really is, because certain complicating influences are almost inevitably present. For example, the percentage of bunt infection in susceptible varieties may be reduced, or even entirely eliminated, by climatic conditions adverse to infection during the seedling stage of the host plant. Classification of the wheat plants depends on the successful development and fruiting of the organism. Mixed physiological strains of the organism may be the disturbing factor in some cases.

Finally, the results obtained with Martin and Hussar indicate that it should be a rather simple matter to use these varieties in breeding for bunt resistance.

Bunt may be controlled satisfactorily in most wheat-growing regions by disinfecting the seed wheat with certain chemicals, but seed treatment should be considered only a temporary means of controlling the disease. Production of resistant varieties suitable for the various wheat regions offers a very promising means for finally eliminating bunt.

SUMMARY

The inheritance of resistance to bunt in wheat was studied in F_1, F_2, and F_3 of crosses of (1) susceptible × susceptible, (2) resistant × resistant, and (3) resistant × susceptible varieties of wheat.

Hard Federation, Baart, and White Federation produced from 50 to 95 per cent of the disease during the five years they were under investigation. During the same period Martin and Hussar were bunt-free.

Hard Federation and Baart differ slightly in their susceptibility to bunt. From a cross between these varieties no plants were isolated which were more resistant or more susceptible than the parents. The difference is probably due to modifying factors.

As contrasted with Martin and Hussar, the three susceptible varieties are considered as completely susceptible.

As compared to the susceptible varieties, resistance to bunt in Martin was completely dominant, and in Hussar was almost completely dominant, as indicated by the F_1.

Martin differs from Hard Federation and from White Federation in one dominant factor for resistance.

Hussar possibly differs from Hard Federation and from Baart in two dominant factors. One of these factors is identical with the factor in Martin. The exact effect of the second factor was not determined.

The factors for resistance to bunt in Martin may be represented tentatively by RRss and in Hussar by RRSS.

On the basis of the results reported in this paper, it should be a simple matter to use Martin and Hussar in breeding for bunt resistance.

LITERATURE CITED

(1) AAMODT, O. S.
1922. THE INHERITANCE OF RESISTANCE TO SEVERAL BIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI IN A CROSS BETWEEN KANRED AND MARQUIS WHEATS. (Abstract) Phytopathology 12: 32.

(2) ———
1923. THE INHERITANCE OF GROWTH HABIT AND RESISTANCE TO STEM RUST IN A CROSS BETWEEN TWO VARIETIES OF COMMON WHEAT. Jour. Agr. Research 24: 457–470, illus.

(3) BARNEY, A. F.
1924. THE INHERITANCE OF SMUT RESISTANCE IN CROSSES OF CERTAIN VARIETIES OF OATS. Jour. Amer. Soc. Agron. 16: 283–291, illus.

(4) BIFFEN, R. H.
1905. MENDEL'S LAWS OF INHERITANCE AND WHEAT BREEDING. Jour. Agr. Sci. 1: 4–48, illus.

(5) BURKHOLDER, W. H.
1918. THE PRODUCTION OF AN ANTHRACNOSE-RESISTANT WHITE MARROW BEAN. Phytopathology 8: 353–359.

(6) CLARK, J. A., MARTIN, J. H., and BALL, C. R.
1922. CLASSIFICATION OF AMERICAN WHEAT VARIETIES. U. S. Dept. Agr. Bul. 1074, 238 p., illus.

(7) FARIS, J. A.
1924. FACTORS INFLUENCING THE INFECTION OF WHEAT BY TILLETIA TRITICI AND TILLETIA LAEVIS. Mycologia 16: 259–282, illus.

(8) FARRER, W.
1901. RESULTS OF THE LAMBRIGG BUNT EXPERIMENTS OF 1900. Agr. Gaz. N. S. Wales 12: 419–430.

(9) ———
1904. REPORT OF THE WHEAT EXPERIMENTALIST. Agr. Gaz. N. S. Wales 15: 1047–1050.

(10) ———
1905. SOME NOTES FOR WHEAT-GROWERS. Agr. Gaz. N. S. Wales 16: 462–472.

(11) GAINES, E. F.
1920. THE INHERITANCE OF RESISTANCE TO BUNT OR STINKING SMUT OF WHEAT. Jour. Amer. Soc. Agron. 12: 124–132.

(12) ———
1923. GENETICS OF BUNT RESISTANCE IN WHEAT. Jour. Agr. Research 23: 445–480, illus.

(13) MCROSTIE, G. P.
1919. INHERITANCE OF ANTHRACNOSE RESISTANCE AS INDICATED BY A CROSS BETWEEN A RESISTANT AND A SUSCEPTIBLE BEAN. Phytopathology 9: 141–148.

(14) ———
1921. INHERITANCE OF DISEASE RESISTANCE IN THE COMMON BEAN. Jour. Amer. Soc. Agron. 13: 15–32.

(15) MELCHERS, L. E., and PARKER, J. H.
1922. INHERITANCE OF RESISTANCE TO BLACK STEM RUST IN CROSSES BETWEEN VARIETIES OF COMMON WHEAT (TRITICUM VULGARE). (Abstract) Phytopathology 12: 31–32.

(16) PYE, H.
1909. WHEAT IMPROVEMENT COMMITTEE. DISEASES AND PESTS OF CEREALS. Jour. Dept. Agr. Victoria 7: 368–373.

(17) REED, G. M.
1924. VARIETAL SUSCEPTIBILITY OF WHEAT TO TILLETIA LAEVIS KÜHN. Phytopathology 14: 437–450.

(18) SUTTON, G. L.
1910. VARIETIES OF WHEAT RECOMMENDED BY THE DEPARTMENT OF AGRICULTURE. Agr. Gaz. N. S. Wales 21: 185–193, 282–288, 593–598, illus.

(19) TISDALE, W. H., MARTIN, J. H., BRIGGS, F. N., MACKIE, W. W., WOOLMAN, H. M., STEPHENS, D. E., GAINES, E. F., and STEVENSON, F. J.
1925. RELATIVE RESISTANCE OF WHEAT TO BUNT IN THE PACIFIC COAST STATES. U. S. Dept. Agr. Bul. 1299, 30 p.

(20) WAKABAYASHI, S.
1921. A STUDY OF HYBRID OATS, AVENA STERILIS × AVENA ORIENTALIS. Jour. Amer. Soc. Agron. 13: 259–266.

THE EFFECT OF OUTSIDE ROWS ON THE YIELDS OF PLOTS OF KAFIR AND MILO, AT HAYS, KANS.[1]

By JOHN S. COLE, *Agronomist*, and A. L. HALLSTED, *Associate Agronomist*,[2] *Office of Dry-Land Agriculture Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The extent to which the yields of plots are affected by the borders or margins of the plots is of much concern to agronomists. The literature on this particular subject, and on the larger subject of the methods of conducting plot experiments, has been reviewed frequently, and a bibliography has been published recently.[3] The literature contains many references to the probable effects of the borders, calculations of the proportion of the borders to the whole in plots of different shapes and sizes, and means of handling plots to bring to harvest a homogeneous unit free from any marginal influence; but it reveals few quantitative determinations of the effect of the border with any crops, and none at all in this country with row crops.

The Office of Dry-Land Agriculture Investigations, Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the Kansas Agricultural Experiment Station, has been conducting crop-rotation and cultural experiments since 1906 at the Fort Hays Branch Station at Hays, Kans. In these experiments the yields are determined from the whole plot. Considerable border effect was frequently observed, particularly in the grain sorghums, which are long-season row crops well adapted to the locality. Investigations were begun in 1914 to determine the extent to which the yields of kafir and milo determined from the entire plot were affected by the outside rows. The plots of these crops already being grown in the rotations and cultural experiments were utilized for this study. On account of the changes in location due to rotation this resulted in differences from year to year in the border conditions of some of the plots—differences that could have been avoided in plots grown especially for the purpose of studying the border effects. The rotations have been described in detail and the results to and including 1920 given in a previous publication.[4]

[1] Received for publication Aug. 28, 1925; issued May, 1926.

[2] The junior writer has had immediate charge of the cooperative work in dry-land agriculture at the Hays station since 1909. All the data reported in this paper were obtained under his supervision. The senior writer is responsible for the calculations and the form in which the data are presented. Both share in the interpretations and conclusions.

[3] WIANCKO, A. T., SALMON, S. C., ARNY, A. C., LOVE, H. H., and MOOERS, C. A. REPORT OF COMMITTEE ON STANDARDIZATION OF FIELD EXPERIMENTS. Jour. Amer. Soc. Agron. 16: 1–16. 1924.

[4] COLE, J. S., and HALLSTED, A. L. METHODS OF WINTER-WHEAT PRODUCTION AT THE FORT HAYS BRANCH STATION. U. S. Dept. Agr. Bul. 1094, 31 p., illus. 1922.

EXPERIMENTAL METHODS

The experimental field is laid out in tenth-acre plots 2 rods wide by 8 rods long, separated on the ends by roadways 1 rod wide and on the sides by alleys 3.3 feet (39.6 inches) wide. A few plots have a roadway on one side. The roadways and alleys are cultivated to keep them free of vegetation except such as grows after crops are laid by.

The plots are plowed, planted, and cultivated lengthwise. Plowing and planting are carried beyond the ends of the plots, and at an early stage of growth the ends are trimmed to the plot lines.

Small grains are sown with a 7-inch drill, with the outside rows either coincident with the edges of the plots or not more than 3.5 inches inside of them. Cultivated crops are planted with 10 rows to the plot, the rows being 40 inches apart. This places the outside rows 18 inches inside of the plot lines. In the work of the Office of Dry-Land Agriculture Investigations, the 40-inch spacing is peculiar to the Hays station. At most stations the rows of intertilled crops are spaced either 44 or 42 inches, bringing the outside rows either to the plot lines or 9 inches inside of them. At Hays the inside rows each have a 40-inch space; outside rows bordering on a roadway or fallow have 20 inches on one side, and, as far as their use of it is concerned, unlimited space on the other; and outside rows adjoining cultivated crops have a space of 20 inches on one side and 37.8 inches on the other, a total of 57.8 inches.

In 1914 and 1915, and from 1919 to 1924, inclusive, the yields of each of 32 to 34 plots of kafir and 7 plots of milo were determined in two fractions. The inside 8 rows of each plot were harvested as one unit and the outside 2 rows as another. The field-cured weights of both grain and stover were determined to the nearest half pound.

The tenth-acre plot consists of 10 rows of equal length, therefore each row is a hundredth of an acre. Eight rows are 0.08 of an acre, and the yield of this fraction is converted to an acre basis by multiplying it by 12.5. Two rows are 0.02 of an acre, and the yield of this fraction is brought to an acre basis by multiplying it by 50. Conversion to some basis of equality is necessary for direct comparison. The acre unit seems the most desirable one to use, although the multiplication of error is very evident.

RESULTS WITH KAFIR

YIELDS OF OUTSIDE AND INSIDE ROWS

Lack of space prevents publication of the data in detail, but the annual average results are given in Table I.

The growth of stalks was good in 1914, but drought interfered seriously with the production of grain. The marginal effect was very marked. The outside rows apparently suffered less from drought, developed more heads, and on every plot made better yields of grain than the inside rows, and on 26 of the 32 plots made higher yields of stover. The average acre yield of grain was 459 pounds on the inside rows and 905 pounds on the outside rows, an increase of 446 pounds, or about 97 per cent. The average increase in the yield of stover was about the same (430 pounds to the acre), but the yields were much greater, the inside rows averaging 4,812 pounds and the outside rows 5,242 pounds. Approximately the

same increases in the yields of grain and stover amounted to nearly 100 per cent in the case of one, and less than 10 per cent in the case of the other.

TABLE I.—*Annual average yields of kafir grain and stover at Hays, Kans., from the entire plots, the inside rows, and the outside rows; the rates per acre of these average yields; and the increases in the rate of yield of the entire plot and of the outside rows over that of the inside rows. (The minus sign indicates a decreased yield)*

Year	Yields per plot (pounds)						Yields per acre (pounds)						Increased yield per acre over 8 inside rows (pounds)			
	Grain			Stover			Grain			Stover			Grain		Stover	
	All 10 rows	Inside 8 rows	Outside 2 rows	All 10 rows	Inside 8 rows	Outside 2 rows	All 10 rows	Inside 8 rows	Outside 2 rows	All 10 rows	Inside 8 rows	Outside 2 rows	All 10 rows	Outside 2 rows	All 10 rows	Outside 2 rows
1914	54.8	36.7	18.1	489.8	384.9	104.8	548	459	905	4,898	4,812	5,242	89	446	86	430
1195	306.5	250.9	55.6	641.1	519.7	121.5	3,065	3,136	2,782	6,411	6,496	6,073	−71	−354	−85	−423
1919	166.4	122.8	43.6				1,664	1,535	2,182				129	647		
1920	352.4	270.7	81.8	732.9	577.5	155.4	3,524	3,383	4,088	7,329	7,219	7,769	141	705	110	550
1921	230.9	174.9	55.9	545.9	426.9	118.9	2,309	2,187	2,797	5,459	5,337	5,946	122	610	122	609
1922	106.9	73.5	31.3	411.0	315.6	95.4	1,069	944	1,567	4,110	3,946	4,768	125	623	164	822
1923	95.6	67.8	27.8	355.3	282.1	73.2	956	847	1,392	3,553	3,526	3,661	109	545	27	135
1924	147.9	101.9	46.0	483.5	377.3	106.2	1,479	1,274	2,298	4,835	4,716	5,309	205	1,024	119	593
Average	182.7	137.7	45.0	522.8	412.0	110.8	1,827	1,721	2,351	5,228	5,180	5,538	106	530	78	388

The precipitation was ample in 1915. Kafir made a heavy crop, but its development was so delayed by low temperatures that it barely matured before frost. The rate of yield of grain from the inside rows was higher than that from the outside rows on all but five of the plots. The average acre yield of grain was 3,136 pounds from the inside rows and 2,782 pounds from the outside rows. The difference of 354 pounds in favor of the inside rows was 11 per cent of their yield. The inside rows had the heavier yield of stover, on all but eight of the plots. The average acre yield of stover was 6,496 pounds from the inside rows and 6,073 pounds from the outside rows. The difference of 423 pounds in favor of the inside rows was 6.5 per cent of their yield.

In 1916, 1917, and 1918 the sorghum crops were delayed by drought, and either did not develop heads or matured little grain. Labor was scarce, so determination of the yields of fractions of plots was not attempted.

Kafir suffered in 1919 more or less continuously from drought after August 16. A small percentage of stalks did not head, and maturity was uneven. The outside rows made higher yields of grain than the inside rows on all but 1 of the 34 plots grown. The average acre yield of grain of the inside rows was 1,535 pounds and of the outside rows 2,182 pounds—a difference of 647 pounds in favor of the outside rows, which was about 42 per cent of the yield of the inside rows. The average acre yield of stover from the entire plots was 2,025 pounds, but the yields of the inside and outside rows were not determined separately.

Although there was some drought near its close, the season of 1920 was so favorable that the yield of kafir was the highest that had been obtained up to that time in the 14 years that the rotation and cultivation experiments had been conducted at Hays. The rate of yield of the outside rows exceeded that of the inside rows on all but two plots in the case of grain, and all but eight in the case of stover. The average acre yield of grain was 3,383 pounds from the inside rows and 4,088 pounds from the outside rows. The difference of 705 pounds in favor of the outside rows was nearly 21 per cent of the yield of the inside rows. The average acre yield of stover was 7,219 pounds from the inside rows and 7,769 pounds from the outside rows—a difference of 550 pounds in favor of the outside rows, which was about 7.6 per cent as much as the yield of the inside rows.

Kafir suffered from drought at different times during the season of 1921, but yielded above the average. The rate of yield of grain from the outside rows was higher than that from the inside rows, on all plots but one, on which it was the same. The average acre yield of grain was 2,187 pounds from the inside rows and 2,797 pounds from the outside rows—a difference of 610 pounds in favor of the outside rows, which was nearly 28 per cent of the yield of the inside rows. The outside rows had the heavier yield of stover on all but four plots. The average acre yield of stover was 5,337 pounds from the inside rows and 5,946 pounds from the outside rows—a difference of 609 pounds in favor of the outside rows, which was about 11 per cent of the yield of the inside rows.

Kafir was prevented from making good yields of grain in 1922 by prolonged drought which began early in July. The growth of stover was less affected, but on those plots that suffered the worst from drought there were few filled heads except in the outside rows. The outside rows had the heavier rate of yield of grain on 31 of the 34 plots, and the heavier yield of stover on the same number. The average acre yield of grain was 944 pounds from the inside rows and 1,567 pounds from the outside rows—a difference of 623 pounds in favor of the outside rows, which was nearly 66 per cent of the yield of the inside rows. The average acre yield of stover was 3,946 pounds from the inside rows and 4,768 pounds from the outside rows—a difference of 823 pounds in favor of the outside rows, which was nearly 21 per cent of the yield of the inside rows.

Kafir was badly damaged by a hailstorm in 1923 when it was about 12 inches high. Following this it was subjected to one of the severest July droughts in 56 years. It survived with sufficient stand and vitality to permit it to respond to later rains and make some fair but below normal yields. The rate of yield from the outside rows was greater than that from the inside rows on all but 5 plots in the case of grain, and 14 plots in the case of stover. The average acre yield of grain was 847 pounds from the inside rows and 1,392 pounds from the outside rows—a difference of 545 pounds in favor of the outside rows, which was about 64 per cent of the yield from the inside rows. The average acre yield of stover was 3,526 pounds from the inside rows and 3,661 pounds from the outside rows—a difference of 135 pounds in favor of the outside rows, which was somewhat less than 4 per cent of the yield from the inside rows.

Kafir suffered in 1924 from intermittent drought from planting to harvest time, and yields were somewhat below the average. The rate of yield of the outside rows was greater than of the inside rows on all plots in the case of grain, and on all but five plots in the case of stover. The average acre yield of grain was 1,274 pounds from the inside rows and 2,298 pounds from the outside rows—a difference of 1,024 pounds in favor of the outside rows, which was about 80 per cent of the yield from the inside rows. The average acre yield of stover was 4,716 pounds from the inside rows and 5,309 pounds from the outside rows—a difference of 593 pounds in favor of the outside rows, which was about 12.6 per cent of the yield from the inside rows.

The 8-year average acre yield of grain was 1,721 pounds from the inside rows and 2,251 pounds from the outside rows—a difference of 530 pounds in favor of the outside rows, which is about 30 per cent of the yield of the inside rows. The yields of stover are available for only seven years. The average yield of stover was 5,150 pounds from the inside rows and only 388 pounds more than that from the outside rows. The yield from the outside rows was only 7.5 per cent greater than the yield from the inside rows.

The percentages that have been given for comparison of the yields from the inside rows with the yields from the outside rows serve some useful purposes, but by themselves they may be more misleading than valuable, because differences in them are so largely fixed by differences in the bases on which they are figured. For example, a difference of 610 pounds in the average yield of grain in 1921 was only 28 per cent, but a difference of 623 pounds in 1922 was 66 per cent.

The general tendency being for the outside rows to yield more than the inside rows, the thought suggested itself that as the yield became higher—approached the maximum yielding power of the crop in any year—the difference between the yields of the two fractions of the plot would become less. If this were true it would be shown by a negative correlation between the yield of the inside rows and the difference in the yield of the outside rows and the inside rows. The question was studied on the yield of grain alone, by constructing dot charts or scatter diagrams, and by calculating the coefficients of correlation.[5]

The first study was made on the 8-year average yields of the 34 plots. The coefficient of correlation between the items under study is -0.30 ± 0.11. The regression equation indicates that the difference by which the yield of the outside rows is greater than that of the inside rows would be reduced to 0 at a yield of 5,063 pounds, or about 84 bushels per acre. The maximum rates of yield that have been observed in this series of determinations were 4,688 pounds from inside rows and 5,100 pounds from outside rows.

A dot chart or scatter diagram of the yield of the inside rows, and the difference in the yield of the outside rows over this quantity, was made for each of the 266 determinations. It indicated a probable negative correlation, although the data were widely dispersed. The coefficient of correlation was calculated and found to be -0.31 ± 0.04. The departures of the individual items from the annual means were used in this calculation.

[5] TOLLEY, H. R., and MENDUM, S. W. A METHOD OF TESTING FARM-MANAGEMENT AND COST-OF-PRODUCTION DATA FOR VALIDITY OF CONCLUSIONS. U. S. Dept. Agr. Circ. 307, 13 p., illus. 1924.

The coefficients of correlation between the two items were 0.32 for 1914; −0.05 for 1915; −0.45 for 1919; −0.33 for 1920; −0.60 for 1921; −0.44 for 1922; −0.60 for 1923; and −0.02 for 1924.

RELATION BETWEEN THE GREATER YIELD OF THE OUTSIDE ROWS AND THE GREATER AREA THEY OCCUPY

It was shown in describing the experiments that the outside rows of many of the plots are outside rows only in the sense of having on one side a wider space between them and the adjoining row than the inside rows have.

The acre yields of grain from the inside rows, and the excess yields of the outside rows over these quantities for each plot each year, were segregated into four groups.

One group was made up of those plots that had kafir on each side of them. There were 154 items in this group (considering corn in one instance as equivalent to kafir). The outside rows in this group had 44 per cent more ground than the inside rows. The average acre yield of the inside rows was 1,804 pounds, and the excess yield of the outside rows over this was 516 pounds, or 29 per cent. If the 23 items that show a lower yield from the outside rows than from the inside rows (17 of which were in 1915) are rejected, and the percentage computed for those that show the greater yield from the outer rows, the 29 per cent rises to 40 per cent. This accords reasonably well with the extra area available to the outside rows.

Another group of 65 items was made up of those plots that had a roadway or fallow on one side. Kafir was the border crop on the other side in 52 of these, and small grain in the other 13. One of the outside rows of each plot in this group had about 44 per cent more space than the inside rows, and the other had unlimited space on one side. The average acre yield of the inside rows was 1,592 pounds. The outside rows yielded at the rate of 2,236 pounds, an excess of 644 pounds (or 40 per cent) over the yield of the inside rows.

The other two groups were smaller. One consisted of 26 plots having kafir on one side and small grain on the other, and the other was made up of 21 plots having small grain on each side. Both were in close agreement with the larger group in which the adjoining plots were kafir, the yields of the outer rows being 29 and 24 per cent greater, respectively, than the yields of the inside rows.

It seems to the writers that this evidence may be of value as a contribution to the data that may be assembled on the problem of how far apart rows should be spaced, but that question is only incidental to the subject of the present paper and so will not be considered further here.

THE EFFECT OF THE OUTSIDE ROWS ON THE YIELD OF THE ENTIRE PLOT

The study so far has been a comparison of the yields of the outside rows and the inside rows of plots. Such comparisons are fundamental, and the results can be applied to plots of any width. The specific question to which the present study is directed is the effect of the outside rows on the yields of entire plots of 10 rows. The basic problem is to determine from the averages of a term of years the relative rank of the several plots representing different cultural or rotation methods, and the values by which the plots are separated

in this ranking. How accurately is this done by using the yields of the entire plot as compared with those of the fraction remaining when the outside rows are rejected?

In the specific problem in hand—the problem presented in the data of the Office of Dry-Land Agriculture Investigations—the outside rows are only one-fifth of the entire plot. The difference between the rate of yield of the entire plot and that of the inside rows consequently is only one-fifth the difference between the rate of yield of the outside rows and that of the inside rows.

The annual acre yields of the entire 10 rows and of the inside 8 rows of each plot were assembled and averaged. This provided 8-year averages for 31 plots, 7-year averages for 1 plot, 6-year averages for 1 plot, and 5-year averages for 1 plot. The averages are given in Table II. This table is arranged in descending order of the yields of the 10-row plots. The 10-row plot is the unit for which it is sought to determine the reliability of the results.

TABLE II.—*The 8-year average rates of yield of grain per acre from the entire 10 rows and the inside 8 rows of 34 plots of kafir at Hays, Kans.*

Rotation or plot	Yields		Rotation or plot	Yields		Rotation or plot	Yields	
	Entire 10 rows	Inside 8 rows		Entire 10 rows	Inside 8 rows		Entire 10 rows	Inside 8 rows
	Lbs.	*Lbs.*		*Lbs.*	*Lbs.*		*Lbs.*	*Lbs.*
C and D	2,488	2,408	558	1,944	1,837	404	1,656	1,542
501	2,316	2,272	508	1,913	1,780	402	1,563	1,401
56	2,185	2,147	559	1,908	1,795	F	1,555	1,484
506	2,140	2,023	E	1,891	1,730	G	1,533	1,480
552	2,133	2,031	507	1,879	1,753	A	1,506	1,365
556	2,108	1,981	555	1,874	1,749	510	1,493	1,385
557	2,079	1,962	553	1,851	1,745	H	1,436	1,260
503	2,070	2,023	551	1,821	1,699	B	1,278	1,188
505	2,039	1,931	560	1,805	1,733	349	1,167	1,015
504	2,028	1,917	401	1,801	1,663	402	1,075	937
55	1,991	1,932	509	1,750	1,645			
502	1,979	1,899	554	1,667	1,558	Average	1,821	1,714

The yields given in Table II are shown graphically in Figure 1. In this figure, the points indicating the rates of yields from the entire plots of 10 rows are connected by a solid line, and those representing the rates of yields from the inside 8 rows by a broken line. The arrangement is in descending order of the yields from the entire 10 rows the same as in Table 2. There are a few breaks in the regular descent of the curve representing the rates of yields from the inside 8 rows, but changes of more than 35 pounds per acre would be necessary on only two plots to bring them all into the order established by the yields of the entire 10 rows. This order places the plot in rotation No. 503 two places lower than the yield of the inside 8 rows places it. A change of 62 pounds per acre in the latter would bring it into the order established by all of the 10 rows. In the same way, the first plot of kafir in rotation No. 402 is two places higher than it would be if arranged in the order of yields from the inside 8 rows, and a change of 85 pounds per acre in the yield of the 8-row plot would be necessary to bring it into the order established by all of the 10 rows. It is believed that none of these minor differences are significant. Rotations Nos. 551, 552, and 553 are duplicates, and

any differences between the yields from them must be attributed to experimental error. In the rates of yields from the inside 8 rows there is a difference of 46 pounds between Nos. 551 and 553, and differences of 332 and 286 pounds between them and No. 552.

The coefficient of correlation between the rates of yields from the inside 8 rows and the entire 10 rows is 0.995 ± 0.001. Critics of the manuscript directed the attention of the writers to the fact that the correlation should be close, because one of each pair of related terms makes up four-fifths of the other. The almost perfect correlation, however, shows that the remaining fifth, the outside two rows, is not a determining, or at least not a disturbing, factor.

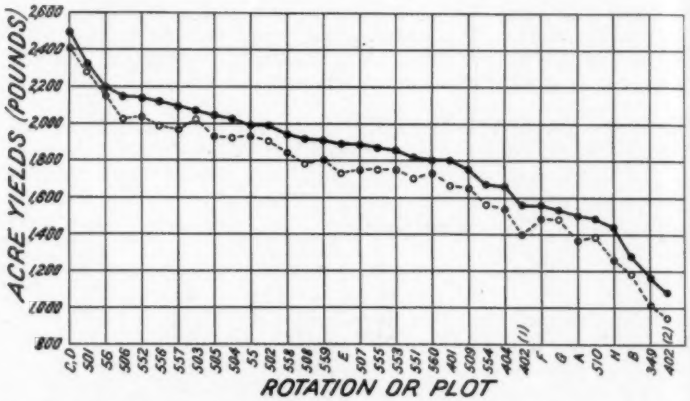


FIG. 1.—Eight-year average yields of kafir grain (Hays, Kans.) from entire plots of 10 rows, shown by dots connected by a solid line. Same for the 8 inside rows shown by dots connected by a broken line

The correlation is shown graphically in Figure 2, which is a dot chart or scatter diagram of the data in Table II. The closeness of the correlation is shown by the closeness with which the dots representing the yields arrange themselves about a straight line. The line in the chart is drawn from the regression equation.

The conclusion from 8 years' investigations with kafir at Hays is that the average yield of 1,827 pounds (or 30.5 bushels) of grain to the acre from the entire 10 rows of the plots is 106 pounds (or 1.8 bushels) higher than the average obtained when the outside rows are discarded (Table I), but that comparisons between methods are not invalidated by the inclusion of the outside rows, nor are they facilitated or made more accurate by their exclusion. The average acre yields of stover were 5,228 pounds from the entire plots, and 5,150 pounds from the inside eight rows. The increase in the yield of stover resulting from including the outside rows amounts to only 78 pounds per acre.

RESULTS WITH MILO

The yields of the outside two rows and of the inside eight rows of seven plots of milo were determined for the eight years that similar determinations were made with kafir. Six of these plots were con-

tinuously cropped to milo, and one was grown on fallow each year. The seasonal conditions were the same as those indicated for kafir.

The results were similar to those with kafir. The outside rows made the heavier yield of grain, except on one plot in 1919, five plots in 1915, and two plots in 1924, when the behavior was reversed. The superiority of the outside rows was not manifested so uniformly in the yield of stover. In 17 of 49 comparisons, the outside rows failed to produce a higher yield of stover than the inside rows.

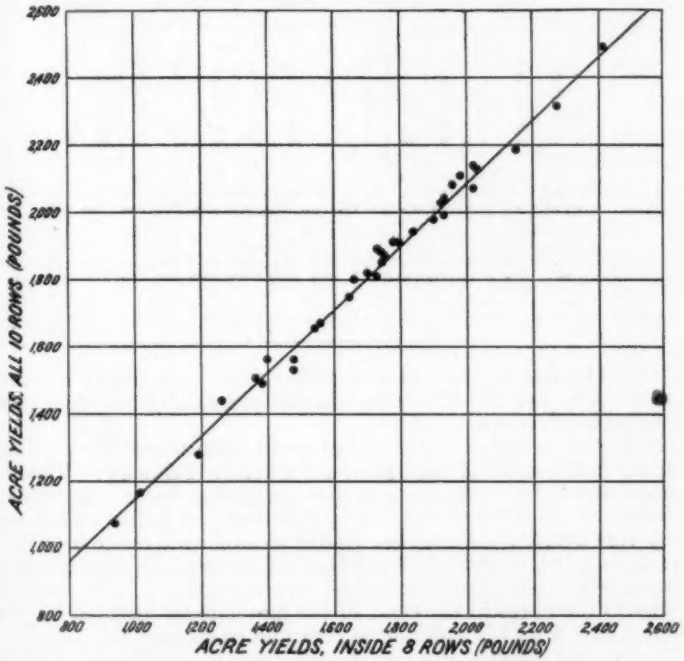


FIG. 2.—Relation between the 8-year average yield of kafir grain (at Hays, Kans.) from the entire 10-row plot, and that from the 8 inside rows

The annual averages, and averages for eight years with grain and seven years with stover, are given in Table III. The average acre yield of grain from the inside rows was 1,449 pounds and from the outside rows 2,071 pounds, an excess of 622 pounds in favor of the outside rows. The average acre yield from the entire plots was 1,573 pounds, or 124 pounds more than the rate of yield from the inside rows.

The average acre yield of stover from the inside rows was 5,184 pounds and from the outside rows 5,601 pounds, an excess of 417 pounds in favor of the outside rows. The average acre yield from the entire plots was 5,267 pounds, only 83 pounds more than the yield from the inside rows.

TABLE III.—*Annual average yields of milo grain and stover at Hays, Kans., from the entire plots, the inside rows, and the outside rows; the rates per acre of these average yields; and the increases in the rate of yield of the entire plot and of the outside rows over that of the inside rows.* (*The minus sign indicates a decreased yield*)

Year	Yields per plot						Yields per acre						Increased yield per acre over 8 inside rows			
	Grain			Stover			Grain			Stover			Grain		Stover	
	All 10 rows	Inside 8 rows	Outside 2 rows	All 10 rows	Inside 8 rows	Outside 2 rows	All 10 rows	Inside 8 rows	Outside 2 rows	All 10 rows	Inside 8 rows	Outside 2 rows	All 10 rows	Outside 2 rows	All 10 rows	Outside 2 rows
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
1914	120.0	86.3	33.7	504.3	405.6	98.7	1,200	1,079	1,686	5,043	5,070	4,936	121	607	−27	−134
1915	236.0	186.1	49.9	786.3	638.9	147.4	2,360	2,326	2,496	7,863	7,987	7,368	34	170	−124	−619
1919	170.7	127.9	42.9				1,707	1,598	2,143				109	545		
1920	274.3	201.1	73.1	778.6	611.0	167.6	2,743	2,514	3,657	7,786	7,638	8,382	229	1,143	149	745
1921	197.1	140.4	56.7	547.1	427.0	120.1	1,971	1,756	2,836	5,471	5,338	6,007	216	1,080	134	669
1922	91.8	66.8	25.0	290.3	221.3	69.0	918	835	1,250	2,903	2,766	3,450	83	415	137	684
1923	48.3	26.4	21.9	530.8	410.6	120.2	483	330	1,093	5,308	5,132	6,011	153	763	176	878
1924	120.3	92.2	28.1	249.7	188.6	61.6	1,203	1,153	1,404	2,497	2,358	3,054	50	251	139	695
Average	157.3	115.9	41.4	526.7	414.7	112.1	1,573	1,449	2,071	5,267	5,184	5,601	124	622	83	417

The differences in the yields of stover were very nearly the same as with kafir, but the differences in the yields of grain were somewhat greater than with kafir.

The 8-year average acre yields of grain from the entire plot, and from the inside rows of each plot, are given in Table IV. The milo plots do not all have the same border conditions, some being flanked by milo on both sides, and others by a roadway or fallow on one side. The 8-year average yields of the 10-row plots show a range from 2,767 pounds to 1,161 pounds, but the rank of the plots is essentially the same as when only the inside rows are considered. The reversals by slight differences in the cases of the methods represented by plots E and A and B and G, can not be regarded as significant.

The relation between the rate of yield from the whole plot and that from the inside rows was studied by determining the correlation of the 56 pairs of variables afforded by the yields of grain from the seven plots in eight years. The coefficient of correlation was found to be 0.99 ± 0.001. This almost perfect correlation between the yields of the individual units tends to confirm the conclusion indicated by the averages.

The 8-year average acre yield of 1,573 pounds of grain obtained by using the yield of the entire 10 rows is 124 pounds higher than the average obtained when the two outside rows are discarded, but the same conclusions as to the relative merits of the cultural methods under study are reached by the use of either unit. The 7-year average acre yield of stover from the 10-row plots was only 83 pounds more than the average rate of yield from the inside 8 rows.

TABLE IV.—*Eight-year average acre yields of milo grain at Hays, Kans., from the entire 10 rows and from the inside 8 rows of each plot*

Plot	Acre yields (pounds)		Plot	Acre yields (pounds)	
	All 10 rows	Inside 8 rows		All 10 rows	Inside 8 rows
C and D	2,767	2,630	G	1,183	1,139
E	1,572	1,410	F	1,161	1,087
A	1,569	1,473			
H	1,514	1,301	Average	1,573	1,449
B	1,246	1,104			

EFFECT OF THE END BORDER

The inside rows are themselves subject to a border effect on each end. This could not be discussed adequately in the introduction without anticipating the conclusions. Observation indicates that on the sides the border effect with such row crops as corn and the sorghums does not extend appreciably beyond the first row. This observation is supported by counts of the number of plants, suckers, and heads in each row. Let it be assumed that the border effect on each end is also exhibited to the depth of one row, which in the present case is 40 inches. The rows are 132 feet long. The end borders, then, constitute 5 per cent of the inside rows. If outside rows yield 25 per cent more than inside rows, and 5 per cent of the area is subject to this effect, the result is an increase of 1.25 per cent in the yield of the whole. The yield of the inside rows, which has been used as a basis of comparison, may average a little more than 1 per cent higher than it would if they were free of border effect on the ends.

DISCUSSION OF RESULTS

Soil-moisture investigations have had a prominent place in the work of which that reported here is a part. The evidence from them and from the rotations and the cultural experiments points to the conclusion that at Hays the higher yields from the outside rows are chiefly a result of the greater quantity of water supplied by the additional volume of soil available to them. The additional quantity of water tends to overcome the effects of drought by extending the time during which water is available. The formation of seed follows the vegetative growth. The value of this extra water supply is manifested more in the yield of seed than in the yield of stover. This generalization of course can not be extended to those cases of drought so extreme that even the outer rows with their additional supply of water are unable to produce seed. Neither would the additional supply of water be effective in increasing the yields in a year when the general supply is so abundant that even the inside rows do not suffer at any time from a lack of it.

It is believed that the results cover a series of years which is sufficiently long to fairly represent the conditions at Hays, Kans., both in their extremes and their averages. Similar determinations should be made in other localities or sections to determine the extent to which outside rows affect the yields under other conditions.

SUMMARY AND CONCLUSIONS

The yields of each of 34 tenth-acre plots of kafir and 7 of milo in the crop rotation and cultural experiments at the Hays Branch Station at Hays, Kans., were determined in two fractions for eight years. One fraction consisted of the outside two rows, and the other of the inside eight rows.

The plots were 2 by 8 rods, and each was planted with 10 rows 40 inches apart. The plants were thinned to about 8 inches apart in the row.

In general, the outside rows yielded more than the inside rows, but the reverse was true in 1915, when temperatures were unusually low and precipitation was unusually heavy.

The excess yield of grain from the outside rows as compared with the yield from the eight inside rows was proportionately much greater than the excess yield of stover from the outside rows as compared with the yield from the eight inside rows.

The 8-year average acre yield of kafir grain was 1,721 pounds from the inside rows and 2,251 pounds from the outside rows—a difference or excess of 530 pounds (or about 30 per cent) in favor of the outside rows. The 7-year average acre yield of kafir stover was 5,150 pounds from the inside rows and 5,538 pounds from the outside rows—a difference or excess of 388 pounds (or 7.5 per cent) in favor of the outside rows.

The 8-year average acre yield of milo grain was 1,449 pounds from the inside rows and 2,071 pounds from the outside rows—a difference or excess of 622 pounds (or about 43 per cent) in favor of the outside rows. The 7-year average acre yield of milo stover was 5,184 pounds from the inside rows and 5,601 pounds from the outside rows—a difference or excess of 417 pounds (or about 8 per cent) in favor of the outside rows.

The excess yield of grain from the outside rows was roughly proportionate to the increased area of soil available to them.

The outside rows constituted but one-fifth of the entire plot. Consequently the rate of yield of the entire plot differed from that of the inside rows by only one-fifth the quantity by which the yield of the outside rows differed from that of the inside rows.

The average acre yield of kafir was 106 pounds of grain and 78 pounds of stover more when determined from all 10 rows than when determined from only the 8 inside rows. Corresponding figures for milo were 124 pounds of grain and 83 pounds of stover.

The relationship between the yields from all 10 rows and the inside 8 rows was linear, the correlation being very nearly perfect. The relative merits of the methods represented consequently could be determined as well by using all 10 rows as by rejecting the outside rows. Yields determined from the entire 10-row plots were, however, subject to a systematic error arising from the fact that the effective areas of the plots were somewhat greater than the conventional areas assigned to them in converting the plot yields to acre yields.

○